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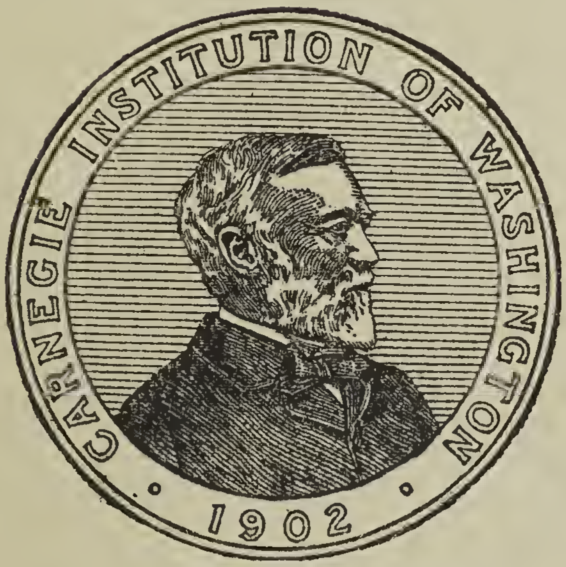

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WASHINGTON, D. C.

CONTRIBUTIONS TO THE GENETICS OF DROSOPHILA SIMULANS AND DROSOPHILA MELANOGASTER

By
A. H. STURTEVANT, C. B. BRIDGES
T. H. MORGAN, L. V. MORGAN
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Published by Carnegie Institution of Washington
Washington, October, 1929

Copies of this book were first issued
October 14, 1929

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I

THE GENETICS OF DROSOPHILA SIMULANS

By A. H. Sturtevant

With one text-figure

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THE GENETICS OF DROSOPHILA SIMULANS

By A. H. Sturtevant

INTRODUCTION

Drosophila simulans is a species of unusual interest to the geneticist, since it is the one form that can be crossed with *D. melanogaster*, and since these are in fact the only two species of the genus that have yet given hybrids. While the hybrids are completely sterile, the fact that *melanogaster* is, by such a wide margin, the organism whose genetic behavior is best known, makes these hybrids of very special interest and importance.

In order to fully exploit the possibilities of the situation, it has been necessary to make an extended study of the genetics of *simulans*. That study has now progressed far enough so that *simulans* is at least as well understood as any other species, aside from *melanogaster*. Mutant genes have been found in each of the four chromosomes, and the maps of the three large chromosomes are well enough developed to make possible the satisfactory study of the numerous special and unexpected problems that have arisen in the course of the work.

The present paper is intended as a summary of what is known about *simulans*, including previously published data and material here recorded for the first time. The mutant types that have been discovered are discussed in alphabetical order; under each one is given a description of the character, comparison with similar types in *melanogaster*, details as to the first appearance of the mutant character, and linkage relations. A summary of all the linkage data available for *simulans* is given. The hybrids with *melanogaster* are described and discussed. Such special topics as the geographical distribution of *simulans*, its chromosome group, and the occurrence in the species of non-disjunction, gynandromorphs, and crossover reducers, are reviewed.

COMPARISON OF *SIMULANS* AND *MELANOGASTER*

External Structure. The two species are very closely similar in appearance. *Simulans* is, however, rather smaller, and stouter in proportion to its length. The wings are relatively shorter than those of *melanogaster*. The "trident" mark, often present on the dorsal surface of the thorax of *melanogaster*, is absent in *simulans;* newly emerged *simulans*, however, have a much more conspicuous dorsal sub-cuticular streak on the thorax. These characters are rather indefinite, and will scarcely suffice to identify individual specimens. The following are more convenient for diagnostic purposes.

The eyes of *simulans* are relatively larger (and the cheeks narrower) than those of *melanogaster*. The anterior filaments of the eggs are longer in *simulans*. The external genitalia of the males are definitely distinct, especially in the shape of the posterior process of the genital arch (hook-like in *melanogaster*, resembling a clam-shell in *simulans*).

Cytology. Adequate cytological material of *simulans* has only recently been obtained, but satisfactory spermatogonial and oögonial preparations are now available. The chromosome group of the female is very similar to that of *melanogaster,* consisting of two pairs of V-shaped autosomes, a small spherical pair (known to correspond to the fourth linkage group), and a pair of rod-shaped X-chromosomes. The only difference noted is that the constriction near the inner end of the X, present in both species, is more conspicuous in *simulans.* The chromosomes of the *simulans* male differ from those of the female in that one of the X's is replaced by a Y. This is quite unlike the Y of *melanogaster* (which is J-shaped and longer than the X), being a straight rod about two thirds the length of the X.

Ephrussi (1925) reported that the mitochondria of the two species were alike in his preparations.

Physiological differences. Very little work has been done on this subject. One difference is clear in the mating preferences of the females, since each species mates more easily with its own male, though both kinds of males seem to court either type of female with equal persistence.

Casual field and laboratory observations suggest differences in food preferences, since *simulans* is rarely found on apples, is relatively commoner on bananas, and seems to be still more frequent on persimmons. No careful study of these preferences has been made.

The development rates of the two species are very nearly the same; but comparisons (made under rather loosely controlled conditions) have several times suggested that *simulans* develops slightly more rapidly. The question needs careful study.

GEOGRAPHICAL DISTRIBUTION OF *SIMULANS*

I have seen specimens of *simulans* from the following localities: Randolph,[1] New Hampshire; Woods Hole,[1] Massachusetts; Cold Spring Harbor,[1] Staten Island,[1] New York; Morristown,[1] New Jersey; Rochester,[1] Minnesota; Richmond, Virginia; Macon, Crescent, Georgia; St. Augustine,[1] Lakeland,[1] Palm Beach, Key Largo, Florida; Kushla,[1] Alabama; Fayetteville, Arkansas; New Orleans,[1] Louisiana; Austin,[1] Texas; Port Limon, Costa Rica; Taboga Island, Panama; Bartica, British Guiana; Corumba (State of Matto Grosso), Saõ Paulo, Brazil. Extensive collections from the West Indies and from the Pacific coast of the United States have not yielded any *simulans,* nor has it been present in any of the European, Japanese, Hawaiian, or Oriental material examined.

The earliest specimen seen was collected in 1912; but it seems clear that Quackenbush (1910) had a stock taken at Woods Hole in 1908. Until 1919 *simulans* would not have been distinguished from *melanogaster,* and accordingly the arguments presented by Johnson (1913) and Sturtevant (1921*c*) to show that *melanogaster* was rare or absent in this country before about 1870 may be applied also to *simulans.* The absence of *simulans* in the older collections so far examined suggests that it was, in fact, introduced even later than *melanogaster*. By 1919 it was nearly as common as *melanogaster* in Florida and New York; but I have always found it much less common at Woods Hole, and its frequency seems to fluctuate greatly from year to year, both in the neighborhood of New York City and in southern Alabama.

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[1] Stocks from localities so marked have been used in the genetic experiments here reported, and have been found to be typical *simulans.* The other records are from preserved specimens.

The present range of the species, as indicated above, suggests that it came from South or Central America. It is not possible to discover whether the spread was a continuous one, through Mexico and Texas, or whether specimens brought from the tropics on fruit boats became established directly in the eastern states; though my own inclination is in favor of the former hypothesis.

HYBRIDIZATION OF *MELANOGASTER* AND *SIMULANS*

The reciprocal matings between *melanogaster* and *simulans* are not equally often successful. From 10 to 40 per cent of the *melanogaster* females mated to *simulans* males produce hybrid offspring, but not over 2 per cent of the *simulans* ♀ × *melanogaster* ♂ matings are successful. Males of each species court females of either species indiscriminately, but neither type of female will accept a foreign male as readily as she will a male of her own species. Apparently, cross-mating regularly results in hybrid offspring, so that the species preferences of the females are the only difficulties in the way of producing hybrids easily. It has been shown (Sturtevant 1915) that yellow females of *melanogaster* are more easily mated with (by *melanogaster* males) than are wild-type females. It is, therefore, not surprising that yellow females of both species have been found to be easier to cross-mate than have wild-type females. In the more difficult cross of *simulans* ♀ × *melanogaster* ♂ this is especially noticeable; most of the successful matings have been from yellow females. Spread plum has also succeeded several times, but wild-type and such mutants as black, ruby, peach, or scarlet have rarely been crossed. These facts indicate that a relatively weak and inactive female is more likely to accept a *melanogaster* male; but experiments designed to weaken females by mutilating legs, wings, or antennæ have not increased the frequency of cross-mating.

Table 1 shows the offspring produced by cross-matings. This table gives all the data obtained from matings in which non-disjunctional exceptions could be detected.

Table 1.—*Offspring of cross-matings between melanogaster and simulans.*

	Regular ♀	Exceptional ♀	Regular ♂	Exceptional ♂	Total
mel. ♀ × sim. ♂...	3698	0	0	440	4138
sim. ♀ × mel. ♂...	400	8	4204	0	4612

The ratio between regular and exceptional offspring in the first cross is not significant, since the *melanogaster* mothers in some matings had attached X's and produced only sons.

As has been pointed out (Sturtevant 1920*b*), analysis of this table indicates that hybrids do not survive unless they carry a *simulans* X, and that survival is usual among those that do carry a *simulans* X, unless a *melanogaster* X is also present and the combination is made in simulans cytoplasm (regular females from the second cross). Under these latter conditions the result is extremely variable. As a rule *simulans* ♀ × *melanogaster* ♂ gives few or no regular females,[1] but some cultures give many of them—at times as many as of the regular males. One large series of matings consistently gave females when the *simulans* mother came from one particular stock, and no females when she came from a certain other stock; but more recent experiments, using the first of these stocks and various other stocks, have given from 1 to 5 per cent of females in every series. This difference in behavior remains unexplained.

Leaving out of account this relative inviability of the regular females from the second mating, there remains an alternative to the interpretation just given (that survival depends on the presence of a *simulans* X): *viz.*, it is possible that hybrids die if they have a *simulans* Y. A decision between these possibilities could be reached if one could determine the fate of the primary exceptional males from the second mating, for they should have a *melanogaster* X and no Y. On the first interpretation they should die, having no *simulans* X; on the second interpretation they should live, having no *simulans* Y. None were present among the 4,204 males recorded; but the number expected is so small that it remains possible that their absence is not significant.

FATE OF REGULAR MALES FROM *MELANOGASTER* ♀ × *SIMULANS* ♂

Melanogaster females were mated to *simulans* males, and were transferred to new cultures each day. When each culture was examined two days later, it was found that the larvæ present could easily be separated into two approximately equal classes according to size. The large larvæ were placed in one culture, and nearly all of them produced adult hybrid females. The small larvæ, placed in another culture, grew slowly. After their sisters had emerged they were still in the larval stage, and were not fully grown. Two of them were dissected by Dr. C. B. Bridges, and were definitely identified by him as males. Only one of these larvæ pupated, and that one formed an elongated abnormal puparium, from which no adult emerged. The others ultimately died, mostly as full-sized larvæ. Superficial examination failed to show any difference in appearance between these and normal larvæ; the cause of their death is accordingly still unknown.

EFFECTS OF TEMPERATURE ON HYBRID FEMALES

It has been found undesirable to keep hybrid cultures (*melanogaster* ♀ × *simulans* ♂) in incubators at 25°C. or higher, since many of the hybrid females are killed at these temperatures. A small series of experiments indicates that from 15° to about 22°C. the hybrids emerge normally and are fully viable. At 25°C. many of them fail to emerge from the puparium, others die when they are half-way out, and many of the remainder die within a few hours after emergence. Such specimens are dark-colored, have shrivelled wings, and are unable to walk normally. At 30°C. the puparia blacken and no adults emerge.

---

[1] The few regular females produced do not come especially often in the later counts from a culture, as was indicated in an earlier account (Sturtevant 1920*b*).

This effect is produced only late in the development of the individual. No exact experiments have been carried out; but a satisfactory routine treatment, that gives no weak blackened specimens, is to keep hybrid cultures at 25°C. until pupation begins, and then remove them to room temperature (20° to 22°C.).

BONNIER'S HISTOLOGICAL STUDIES OF THE HYBRIDS

Bonnier (1924) has reported some results of histological studies of hybrids from *melanogaster* ♀ × *simulans* ♂. Three results may be noted here.

1. The chromosomes of the hybrid females resemble those of the parent species, and the somatic pairing characteristic of the Diptera is present. This result is clearly important; but unfortunately it is based on a single oögonial division-figure, the drawing of which is not convincing. The result is, however, what other data would lead one to expect.
2. Three hybrid larvæ (presumably males) are described. Bonnier concludes that the different organs develop at different and variable rates. Some of the hybrid larvæ appeared "perfectly normal" so far as studied, and the number of control specimens seems to have been small. It seems possible that the abnormalities found in the three larvæ described in detail were not due to the fact that they were hybrids.
3. A study of the ovaries of hybrid females indicated that degeneration of the oögonial tissue regularly sets in before synapsis and the deposition of yolk would be expected, and that these two processes do not occur. As in some other described species hybrids, sterility is not directly due to failure of unlike chromosomes to conjugate, but to degenerative processes that set in before the time of conjugation. Bonnier reports that the development of the hybrid ovaries is variable, which agrees with my own observations on dissected adult females.

BRISTLES OF THE HYBRIDS

The various classes of hybrids are all known to have missing bristles at times, but the study of this phenomenon has been limited almost entirely to the hybrid females from *melanogaster* ♀ × *simulans* ♂.

At least two major factors influence the frequency of missing bristles, *viz.*, age of culture and modifying genes present in the mother. In the case of most mutant types of bristle reduction, the flies emerging early in the history of a culture have more bristles than those that come later; in the case of the hybrids this relation is reversed. It is clear on inspection that the later hatched flies have more bristles, but specific data have been collected on a few cultures. The results were studied by determining the correlation coefficient between number of dorsocentral bristles and day of counting. In eleven cultures this coefficient ranged from −.026±.076 to +.470±.040 (positive in all but the one case just noted), with an average of +.217. This result is due to aging of the culture medium, not of the mother, as was clearly shown by transferring parents to a new culture bottle. Under these conditions the new culture starts at the same low point as the first one did, not at the high point at which the first one ended.

The average number of bristles on the hybrids is also dependent on modifiers, though the data indicate that in general the *simulans* stocks used were equivalent (excluding hairless, the dominant bristle-reducing mutant type, from consideration). Several *melanogaster* stocks tested gave characteristically different results, regardless of the source of the *simulans* male that was used. The three most extensively studied stocks are indicated in table 2.

TABLE 2.

Melanogaster stock	Number of hybrid cultures	Extremes—mean number of dorsocentrals	Average
dachs	7	3.5 to 3.9	3.67
peach spineless sooty rough	5	3.5 to 3.7	3.61
black purple vestigial arc speck	5	2.0 to 2.4	2.22

One might suspect that these results were due to cytoplasmic inheritance, since the race from which the father comes is of no significance. The following experiment shows that such is not the case. The two multiple recessive stocks of table 2 were crossed, reciprocally, and the F_1 females were mated to *simulans*. From (peach spineless sooty rough × black purple vestigial arc speck) female × *simulans* male, 245 hybrid females were obtained, with a mean dorsocentral number of 2.637±.057. From (black etc. × peach etc.) female × *simulans* male, there were 352 hybrid females, and the mean number of dorsocentrals was 2.693±.045. The difference between the two means is less than its own probable error (.056±.073), and is in the direction opposite to that required for a cytoplasmic interpretation. The data, of course, do not eliminate the possibility of a maternal effect due to genes.

The modifiers for bristle number have not been located. It has, however, been shown that the dominant mutant dichæte is not affected by the same series of modifiers. Females of some of the stocks tested for effect on hybrid bristle number were mated to dichæte males from one particular stock.

The dorsocentrals of the resulting F_1 flies were counted, with the results shown in table 3.

There is clearly no correlation between the two series of results. This was perhaps to have been expected, since a study of the pattern of bristle reduction shows that the hybrids differ markedly from most of the mutant types.

The data collected by Plunkett (1926) show that such mutant types as dichæte or hairless are relatively specific in their effects. Both these types, for example, often lack an anterior dorsocentral but much more rarely lack a posterior one. Neither is likely to lack a scutellar bristle; but the mutant scute nearly always lacks one or more scutellars, while it rarely affects the dorsocentrals. These relations do not hold in the hybrids. A total of 167 hybrid females were examined for dorsocentrals and for scutellars. A total of 142 anterior dorsocentrals were present (i. e., 2 × 167 − 142 = 192 were missing). There were 181 posterior dorsocentrals (153 missing), 192 anterior scutellars (142 missing), and 198 posterior scutellars (136 missing).

Table 3.

Stock	Hybrid females	Dichætes
yellow white	3.72	0.60
peach spineless sooty rough	3.61	1.13
bar	3.39	0.64
black vestigial	3.10	2.25
black purple vestigial arc speck	2.22	1.58

The differences here are significant in certain cases (*i. e.*, the anterior dorsocentral is more often absent than are the other bristles named); but the differences are much less in extent than in the case of any mutant type yet recorded.

If allowance is made for genetic and environmental modifiers, analysis shows that there is no positive correlation between bristle-numbers on the two sides of the body. Plunkett (1926) has already reported the same thing for the mutant dichæte. In both cases, both sides of the body are affected alike by genetic and environmental modifiers, so that there is a strong positive correlation between the two sides in ordinary mixed series. In the case of the hybrids the modifiers were made uniform by taking into account only those specimens in which two and only two dorsocentrals, scutellars, or notopleurals were present (two on each side of the body being the normal condition). Of 185 hybrids with two dorsocentrals, 130 had both on the same side of the body; of 101 with two scutellars, 81 had both on the same side; of 101 with two notopleurals, 72 had both on the same side. The corresponding numbers for mutant types are very different. Of 223 scute flies with two scutellars, only 10 had both on the same side; of 29 dichætes and 60 hairless with two dorsocentrals, none had both on the same side of the body. This difference is not due to a difference in right-left correlation: as just stated, neither the mutants nor the hybrids show such a correlation under these conditions. But in the mutant types there is not only a greater specificity of action, but also a directional effect. In dichæte, for example, the anterior dorsocentral is absent much more often than the posterior—and the posterior is never absent if the anterior of the same side is present. Similar relations hold in hairless and in scute; but in the hybrids either anterior or posterior may be absent when the other is present. It is apparently this relation that is responsible for the different proportions among the two-bristled specimens.

The results just discussed can only mean that a large portion of the variability in bristle number of the hybrids is due to influences that act separately on the two sides of the body. Much time has been spent in an attempt to analyze the patterns further. It appears that there are localized influences within one side of the body, but no fully satisfactory statistical treatment of the data has yet been devised. The present indications are that the cell-lineage of the tissue concerned shows a correlation with the distribution of affected bristles; but that this correlation is not high enough to make it probable that cell-lineage is the controlling factor. It seems more probable that some sort of bristle-inhibiting influence spreads from certain centers (*see* Plunkett 1926), and that the position of these centers is relatively constant in the mutant types, much more variable in the hybrids.

MUTANT TYPES STUDIED IN *SIMULANS*

ABNORMAL-WING (a-w; II, 52)

Description. The wings of this type vary from a shape indistinguishable from wild-type to forms suggestive of miniature, of rudimentary or truncate, or of bent in *melanogaster.* The two wings of a specimen are often unlike. A bending posteriorly near the base, a shortening of the whole wing, and a narrowing due to the decreased convexity of both margins are perhaps the commonest characteristics, but bluntness of the apex is also frequent. The viability and fertility of the type are good, but the fact that it overlaps wild-type renders it of little use in linkage experiments. The character is recessive.

No closely similar character is recorded in *melanogaster,* and abnormal-wing has not been tested in hybrids.

Origin. A single male of the abnormal-wing type was found (November, 1924) in ruby stock, of pure Florida ancestry. No analysis of his origin is possible.

Preliminary experiments showed that a-w was independent of hairless (third chromosome) in the male, and that F_2 from black (second chromosome) by abnormal-wing included no black abnormal-wing specimens. The gene was thus shown to lie in chromosome-II.

ARC (ar; II, 97 or 109)

Description. In pure *simulans* arc is a recessive lethal, with no observable somatic effects. It is accordingly of little use for linkage purposes, and has been discarded.

Comparison with Melanogaster. As will appear in the following section, arc was first discovered through the fact that it is allelomorphic to *melanogaster* arc.

Origin. In December, 1920, a black purple vestigial arc speck strain of *melanogaster* was crossed to a peach strain of *simulans* derived from a wild stock collected at Kushla, Alabama, in April, 1920. Several of the hybrid offspring had arc wings, quite like those of pure *melanogaster* arc. Careful search failed to show any arcs in the peach *simulans* stock; but further crosses of this stock to arc *melanogaster* again gave some arc hybrids. Accordingly, four males from the peach stock were mated, individually, to arc *melanogaster.* Two of these matings gave only wild-type hybrids; in each of the other two, half of the hybrids were arc. The two males thus shown to be heterozygous for arc were then remated to plum females, and sons from these matings were crossed, individually, to arc *melanogaster*. More than 20 such matings were made, but offspring were produced from only two of them; one of these gave no arc hybrids, the other gave 39 wild-type hybrids and 47 arcs—all females. The father of this second culture was then $\frac{\text{pm}}{\text{ar}}$ in constitution. He was remated to plum females, and his not-plum second chromosome was kept for many generations (depending always on the absence of crossing over in males).

Matings of $\frac{\text{pm}}{\text{ar}}$ ♀ × $\frac{\text{pm}}{\text{ar}}$ ♂ gave no arc offspring, but did give approximately a 2 : 1 ratio for not-plum to plum (*see* page 32, under semi-forked), indicating the presence of a lethal in the arc chromosome. All the data are consistent with the view that this lethal is in fact ar itself. The locus of ar was studied by crosses to arc *melanogaster*. Females of the constitution $\frac{\text{ar}}{\text{sd pm}}$ were mated to sd pm males. Wild-type sons and plum sons from this mating were then mated, individually, to arc *melanogaster* females. Of the 14 wild-type males so tested, 13 gave arc hybrid offspring; of the 16 plums, one gave arcs. It follows that arc is roughly 7 units from plum. One of the two cross-overs observed must have been a double cross-over, but it is not possible, from these data, to judge which one was. That is, arc is near plum but may lie either to the right or to the left of it. In drawing the map, I have arbitrarily put the arc locus to the left of plum.

ARISTAPEDIA (ap; III, 75±)

Description. The arista resembles a tarsus, having more or less distinct segments, definite hairs of the usual black type found on the legs, and two terminal claws quite like those of the tarsi. The third antennal joint is abnormally shaped, bears black hairs, and the arista arises further from its base than in wild-type. The two basal antennal segments are somewhat elongated. All these characters serve to make the antenna approach a leg in appearance. The legs themselves are modified, the four terminal tarsal segments being very short, sometimes with no divisions between them so that the tarsus appears to have only two segments. The basal tarsal segment always looks normal, and sex-combs are always present on the front legs of males. Claws are also always present at the apex of the tarsus. The viability of the type is poor, and both sexes are occasionally sterile though usually fertile. The character is not noticeably variable, and is always sharply separable from wild-type. It is completely recessive.

Comparison with Melanogaster. Professor S. S. Chetverikov has discovered a *melanogaster* mutant type that has antennæ closely similar to those of *simulans* aristapedia. The tarsi of the *melanogaster* type are normal, but the bristles of the whole body are somewhat reduced in size (not so in *simulans* aristapedia). My experiments show that *melanogaster* aristapedia occupies the same locus as the well-known type spineless, and that ap/ss flies have small bristles. Crosses show that *melanogaster* aristapedia and *simulans* aristapedia are both recessive in the hybrids; and that hybrids heterozygous for both genes have the characteristic leg-like antenna, but normal tarsi and bristles. *Simulans* aristapedia is therefore allelomorphic to *melanogaster* aristapedia, which, in turn, is at the spineless locus.

Origin. Aristapedia appeared, November, 1925, as numerous males and females in a small mass culture that formed part of a series selected for extra scutellar bristles. This strain had been inbred for 10 generations; it came originally from a cross of claret by yellow white black, and was largely of Morristown (N. J.) and Kushla (Ala.) ancestry.

BIPECTINATE (bp; III, 78)

Description. The name of this form refers to the presence of two sexcombs on each front leg of the male—one on each of the two basal tarsal segments, as in normal males of *Drosophila obscura.* Characters observable in both sexes are: one tarsal segment absent on every leg, and wings short, broad, and held out from the body. Bipectinate flies are poorly viable, do not live well after emergence, and often fail to breed. They are always perfectly separable from wild-type, but the difficulty of keeping a pure stock was so great that the type was discarded. The character is recessive.

Origin. Numerous bipectinate individuals were found, October, 1921, in a wild stock that came from Rochester, Minnesota.

BLACK (b; II, 43)

Description. The whole body is somewhat darker than that of wild-type, especially on the dorsal surface of the head and thorax. The wing veins are bordered by dark shadows. The type differs from most of the dark mutant forms of *melanogaster* in having no well-marked black pattern on the dorsal surface of the thorax—a difference evidently correlated with the complete absence of such a pattern in the wild-type and haplo-IV *simulans. Simulans* black darkens considerably with age, but is often difficult to classify in young flies. This difficulty is increased by the fact that flies heterozygous for black are slightly darker than wild-type. Nevertheless, with sufficient care and experience it is possible to make complete and accurate classifications when dealing with black.

Yellow black flies are brownish in color, easily distinguishable from wild-type or from black, but less easily separable from yellow than is black from wild-type. Black straw shows similar relations; it is brownish, distinct from wild-type and from black, but not easily distinguishable from straw. Here again, however, care and experience make classification possible and accurate.

Comparison with Melanogaster. Black *melanogaster* has been shown to be recessive in crosses to wild-type *simulans.* Black *simulans* has been crossed to wild-type, sable, and black *melanogaster:* the first two types of matings give wild-type, the black × black mating gives hybrids intermediate in color between the two parents. *Simulans* black is therefore allelomorphic to *melanogaster* black.

Origin. Many black individuals were found (November, 1921) in a wild stock that had recently been collected at Morristown, New Jersey. It is probable that the mutation occurred before the strain was brought into the laboratory.

BOBBED (bb; I, 66.7)

Description. Bobbed has bristles that are shorter and finer than those of wild-type, and often has abnormal abdominal bands that are broken and are less heavily pigmented than in wild-type. The more extreme types develop more slowly than wild-type. There is a marked sexual dimorphism, the females only rarely showing the character well enough to enable one to separate them from wild-type. The males, though regularly more extreme than sister females, are variable and in some cultures are not easily identified. Crosses of bobbed males to claret females yield XO bobbed males that are no more extreme than bobbed females, showing that the usual sexual dimorphism is due to an intensification of the character by the Y-chromosome. The nature of the character in XXY bobbed females and in XYY bobbed males is difficult to study, owing to the low frequency of secondary non-disjunction in *simulans,* and to the fact that bobbed so often overlaps wild-type in XX females and in XY males. These experiments are still in progress, but have not yet yielded definite results. The effects of Y's from different sources have been studied by mating bobbed females to males from various wild stocks (or uncrossed mutant descendants of wild stocks). The following were found to have Y's that intensified the bobbed character in males: four from Kushla, Alabama (1916, 1920, 1921, 1924), and one each from New Orleans, Louisiana (1926); St. Augustine, Florida (1923); Morristown, New Jersey (1921); Rochester, Minnesota (1919); Lakeland, Florida (1919); Woods Hole, Massachusetts (1922). In the Woods Hole stock, however, there were also present Y's that were without effect on bobbed. Bobbed XY males with this Y resemble XO bobbed males, and a pure stock of bobbed in which only this Y is present shows no sexual dimorphism in the expression of the bobbed character.

Bobbed is not a favorable character for linkage experiments, because it overlaps wild-type and because it hatches late when the character is well-developed. Because of its terminal position in the map, it is nevertheless sometimes necessary to use it. One favorable circumstance in this connection is that its classifiability is not interfered with by any other gene in the right end of the X, though forked might have been expected to have such an effect. The size of the bristles is relatively as much reduced in forked bobbed as in not-forked bobbed, and the abnormal abdominal bands are not at all influenced by forked. Bobbed is fully fertile, even when the character is present in extreme form. It is completely recessive.

Comparison with Melanogaster. Bobbed *melanogaster* resembles bobbed *simulans,* but the best-known allelomorph in *melanogaster* is more extreme than that of *simulans,* producing also relatively more effect on the bristles and relatively less on the abdomen. As Stern (1927*a*) has shown, the Y of *melanogaster* normally carries a suppressor of bobbed rather than an intensifier like that of the *simulans* Y. As a result a pure stock of *melanogaster* bobbed is sexually dimorphic in the reverse direction from that found in *simulans*—the females show the character but the males appear wild-type. In each species the locus is the rightmost known one in the X.

Each type has been found to be recessive in hybrid females. Bobbed *melanogaster* female × bobbed *simulans* male gives bobbed hybrid females, showing that the genes are allelomorphic. Attached-X *melanogaster* females by bobbed *simulans* males give hybrid males that have a bobbed *simulans* X and a *melanogaster* Y carrying a bobbed suppressor. Such males are wild-type as to bristles, as might have been expected, and have abdomens no more abnormal than is usual for not-bobbed hybrid males.

Origin. A single bobbed male was found November 6, 1922, in a wild stock that was collected at Kushla, Alabama, in 1921. This male was mated to ruby dusky females, and produced wild-type daughters and ruby dusky sons. Three of the wild-type daughters were tested, and all gave the results expected from $\frac{\mathrm{rb\ dy}}{\mathrm{bb}}$ females. This strain of bobbed was lost after it had been thoroughly tested, but the character has since reappeared.

In December, 1926, it was found that a female that was heterozygous for the third-chromosome genes sepia, scarlet, hairless, and aristapedia was producing sons about half of which were bobbed. Tests have shown that this is a true bobbed, allelomorphic to *melanogaster* bobbed and occupying the same locus as the old *simulans* bobbed, which it closely resembles. The pedigree of the female that produced the second series of bobbed flies is very complex, but does not trace to the Kushla 1921 stock that gave rise to the first bobbed mutation, so there must have been two separate mutations, even though the frequent overlapping of wild-type by bobbed makes it impossible to be certain in either case that the mutation had not occurred many generations before it was detected.

A third bobbed was found October 25, 1927. A plum female, heterozygous for claret, was mated to a dusky forked claret male. There resulted 47 daughters and 39 sons; two of the sons (not dusky, not forked, and therefore not due to non-disjunction) showed the bobbed character. Here again the pedigree of the mother is very complex. She was remotely related to the mother of the original bobbed-2 series, and she also traced to the Kushla 1921 stock that produced bobbed-1. But both these relationships are so remote that it is practically certain that a third mutation to bobbed occurred in this strain. Bobbed-3 closely resembles the other two types; linkage tests have shown that it lies well to the right of forked, and when crossed to bobbed *melanogaster* it gave bobbed hybrid females.

BRANCHED (II, III)

In March, 1920, several flies were found in which the apex of the fifth vein was branched. As previously reported (Sturtevant 1921), tests showed this to be a variable character, dependent on genes in the second and in the third chromosome. Apparently all flies showing the branched character are homogygous for branched-III, and most of them are homogygous for branched-II. Branched has been discarded. The genes concerned were never accurately located.

CLARET (ca; III, 134±)

Description. The eyes of claret are of a translucent yellowish pink, very similar to those of peach, ruby, or garnet *simulans* and to the four *melanogaster* types bearing these same names. Scarlet claret is yellowish, easily separable from either single mutant type. Plum claret is not noticeably different from claret. White claret is white in color. Claret is recessive, and has excellent viability. The males are fully fertile.

As described more fully in a paper now in press, claret females are often sterile, and never produce many offspring, though they lay many eggs. The offspring that are produced include many unexpected classes. About half the males are primary non-disjunctional (XO) exceptions to the usual type of sex-linkage, and about 6 per cent of the females are also non-disjunctional exceptions (XXY). More than 3 per cent of all the offspring produced by claret females are gynandromorphs, in which a maternal X-chromosome has been eliminated during a cleavage division. About 40 per cent of all the offspring are diminished (haplo-IV), and an additional 4 per cent are diminished mosaics (haplo-IV in part but not all their bodies).

These irregularities are all consistent with the view that, in the oögonia and oöcytes of claret females and in the cleavage divisions of their offspring, the rate of division of the chromosomes (only the maternal chromosomes in the cleavage divisions) is slowed down in relation to the rate at which the rest of the mitotic mechanism functions. This retardation results in chromosomes occasionally failing to pass to either pole at division (elimination), or, more rarely, in both homologues passing to the same pole (non-disjunction). The cases in which this happens to the first or fourth chromosomes give the exceptional classes noted; when it happens to the second or third chromosomes the resulting zygote is presumably inviable, and this is the reason for the high mortality in the egg stage among the offspring of claret females.

Comparison with Melanogaster. *Melanogaster* claret has been found to be recessive in hybrids; *melanogaster* claret × *simulans* claret gives claret hybrids. The two clarets are therefore allelomorphic. Numerous attempts to cross claret *simulans* females × *melanogaster* males have given no results.

Origin. Claret was discovered in 1924, by Dr. C. R. Plunkett, in a stock from Austin, Texas. This stock was found to be contaminated at the time, so it is not quite certain that the claret mutation occurred in it.

CROSSVEINLESS (cv; I, 14.0)

Description. Both crossveins are usually missing, though there is sometimes a suggestion of the anterior one still present. There is a slight thickening of the apices of the second to fourth longitudinal veins, especially where they meet the marginal vein. The viability of the type is good, and both sexes are quite fertile. The character is recessive. It has been lost, and is therefore not now available for experiments.

Crossveinless was studied with most of the other sex-linked mutant types, and gave no unusual interaction effects except with fused. Crossveinless fused flies show both characters, and neither is exaggerated; but their viability is very greatly reduced. This is perhaps expressed most simply by saying that crossveinless acts as a disproportional intensifier of the relatively low viability always characteristic of fused.

Comparison with other species. The crossveinless character closely resembles those of the same name in *melanogaster, virilis,* and *willistoni.* These are all sex-linked types, and in *melanogaster* the locus (13.7) is near that of *simulans* crossveinless. There is, however, no marked effect on the viability of fused in *melanogaster.*

Both *melanogaster* and *simulans* crossveinless have been found to be recessive in hybrids between the two species, and crossveinless *melanogaster* ♀ × crossveinless *simulans* ♂ has given only crossveinless daughters. The two are therefore allelomorphic genes. *Simulans* crossveinless produces its characteristic effects in hybrid males.

Origin. A single crossveinless male was found, January 9, 1924, in a wild stock that had been collected at Morristown, New Jersey, in 1923. This male was mated to yellow prune, and later to ruby dusky females. The four daughters that were tested were all heterozygous for crossveinless.

CURVED-LIKE (cu; III, 0)

Description. The wings are held out at right angles to the body and are curved downwards. The character is definitely different from the curved of *melanogaster,* in that the wings are never at less than a 90° angle from the long axis of the fly (usually less than such an angle in curved), and in that the wings are curved along their long axis but not along their short axis. The character is of poor viability, and has accordingly been discarded, despite its perfect separability and desirable locus. It is recessive.

Origin. Curved-like appeared (April, 1921) in a mass culture belonging to the early experiments with peach and rugose, of Alabama and Florida ancestry.

DACHS-LIKE (d-l; III, 28±)

Description. As already stated (Sturtevant, 1921*b*), this character closely resembles the dachs of *melanogaster.* The legs are short, the tarsi being four-segmented. The wings are somewhat shortened, the second vein relatively less so than the rest of the wing. The flies are usually small, and their viability and fertility are poor. The character is recessive. It has been discarded.

Comparison with Melanogaster. As stated in the earlier account, hybrids with one *simulans* d-l and one *melanogaster* dachs are wild-type in appearance.

Origin. Dachs-like was found (September, 1920) by Dr. T. H. Morgan in a yellow forked stock, of pure Florida ancestry. It was found at this time to be widely scattered in descendants of this yellow forked stock.

DASH (d; II, 5±)

Description. There is a break in the last section of the fourth vein, which is variable in extent. Sometimes nearly the whole section is absent, sometimes there is no actual break but only a thin portion of the vein, and sometimes there is no indication of the character at all. The viability and fertility of dash are good, but the fact that it overlaps wild-type limits the usefulness of the type. Dash is recessive.

Comparison with Melanogaster. Dash is recessive in hybrids, and the similar character of the same name in *melanogaster* is also recessive in hybrids. Dash *melanogaster* by dash *simulans* gives hybrids many of which show the dash character. Dash *melanogaster* is dependent on two genes, and the loci of these have not been satisfactorily determined, so that the dash locus of *simulans* cannot be correlated with the *melanogaster* map until more experiments are carried out.

Origin. Several dash flies appeared, February, 1924, in the F_2 generation from garnet forked females mated to the original fused mutant male. Examination of the stock from which this fused male came showed that dash was present in it. The mutation evidently occurred in an earlier generation in that stock.

DELTA (Δ; III, 64)

Description. This is the character previously called deltoid, the delta of earlier accounts (Sturtevant 1921*b*) having been renamed delta-like (q.v.). Delta makes the wing-veins thicker than wild-type, with irregular knot-like thickenings and with delta-like spots at most of the junctions of veins. The acrostichal hairs are also more irregular than in wild-type, and in more than the usual eight rows. Delta is dominant, and is lethal when homozygous. Its viability and fertility are excellent, and it is always separable from wild-type. Delta and hairless counteract each others effects in part, in that delta hairless flies have more normal venation than delta, and more nearly the normal number of bristles than hairless. Classification is, however, never doubtful.

Comparison with Melanogaster. Delta resembles *melanogaster* delta, but is less extreme. Each is dominant in the hybrids; the hybrids with *melanogaster* delta are less extreme than pure *melanogaster* with delta, still less extreme are the hybrids with *simulans* delta, and least extreme (*i. e.*, nearest wild-type) are the pure *simulans* deltas. It follows that *melanogaster* delta is more extreme than *simulans* delta, and that the modifiers present in *melanogaster* also intensify the delta character more than do those of *simulans*.

As previously recorded (Sturtevant and Plunkett 1926), the allelomorphism of delta and of hairless was tested in one series of experiments. *Melanogaster* females $\frac{\Delta}{H}$ × *simulans* males $\frac{\Delta}{H}$ gave 169 hybrids: 164 delta hairless, 4 delta, 1 hairless, 0 wild-type. The 4 delta and 1 hairless are due to crossing over in the mother between Δ and H (known to occur in 3 per cent of the eggs). *Melanogaster* females $\frac{\Delta H}{+}$ × *simulans* males $\frac{\Delta}{H}$ gave 1 delta hairless, 225 delta, 226 hairless, 0 wild-type. There was a crossover inhibitor present in the mother, so the one delta hairless here was unexpected. It remains unexplained, but was presumably due to mutation or to a rare crossover (such should occur in a very small number of the eggs). The results of these two crosses are intelligible only on the assumption that both $\frac{\Delta}{\Delta}$ and $\frac{H}{H}$ hybrids die. That is, the two deltas and the two hairless genes, respectively, are allelomorphic.

Origin. A single delta female was found by Dr. C. B. Bridges (December 31, 1920) in the peach stock, descended from a wild stock collected in May, 1920, at Kushla, Alabama. Mated to wild-type males, this female produced 40 delta offspring and 57 wild-types.

DELTA-LIKE (III)

This type was described and figured (Sturtevant 1921*b*) under the name delta, now applied to a different type (q.v.). Delta-like resembles delta, but is rather more extreme. It is dominant, and the females are sterile. Owing to the latter peculiarity the locus could not be determined, and the stock was accordingly discarded.

Delta-like was found (December, 1919) as a single male from a pair mating of pure Florida ancestry.

DUSKY (dy; I, 36.2)

Description. Dusky has wings much shorter than those of the wild-type, slightly darker in color, but of nearly the normal shape and proportions. The wings are held out from the body somewhat in some specimens, and rather frequently have a few small marginal notches near the apex. The character is always easily separable from wild-type. Both sexes are fully fertile, and the viability is usually good but at times is rather poor in flies that are also yellow. Dusky is recessive.

Comparison with Melanogaster. Dusky is more extreme (*i. e.*, produces smaller wings) than either of the dusky allelomorphs in *melanogaster,* and is almost as extreme as miniature *melanogaster.* Dusky *simulans* males were mated both to miniature and to dusky *melanogaster* females: the first of these matings gave wild-type hybrid females, the second gave hybrid females intermediate in appearance between dusky *melanogaster* and dusky *simulans.* The two are therefore allelomorphic.

Origin. In a culture pure for the third-chromosome eye-color scarlet, a single male was found that had a dusky left wing, and a wild-type right wing. This male was mated to ruby females. There resulted 75 wild-type females, 65 ruby males, 2 dusky males, and 1 wild-type male (culture 10,726). The three not-ruby males were evidently due to non-disjunction and each received a paternal X. Since two of these were dusky and one was not, the germ-cells as well as the soma of the father of 10,726 must have been partly dusky, partly not. Seven of the wild-type daughters of 10,726 gave the results expected from $\frac{\mathrm{rb}}{\mathrm{dy}}$ females; three other daughters, tested in one culture, gave 129 daughters, 42 wild-type sons, 54 ruby sons, 40 dusky sons, and 7 ruby dusky sons. It seems clear from this result that at least one of these three females carried dusky and at least one did not. The total tested X-bearing sperms of the original mosaic male were, therefore, 10 dusky and 2 not-dusky.

No further mutations in the dusky locus have been detected in *simulans.*

DWARF (dw; I, 3.1±)

Dwarf has already been described and its origin and relation to *melanogaster* given (Sturtevant 1921*a*). The character, which has been discarded, was usually lethal. The few survivors were very small but otherwise appeared normal, and both sexes were fertile. It resembled lethal-10 of *melanogaster,* but was probably not allelomorphic to it. Dwarf was first detected through a 2 : 1 sex-ratio given by a single female heterozygous for yellow, most of whose sons were yellow.

EXTENDED (E; I, 3.0)

Description. This is the "Gull" of Morgan, Bridges, and Sturtevant (1925). The wings are extended at about 90° from the long axis of the body, are rather broad at the apex, and are slightly convex as viewed from above. The acrostichal hairs are in irregular rows and are more numerous than in wild-type flies. The character is dominant, and is lethal in males—that is, it behaves like the well-known notch of *melanogaster, obscura,* or *funebris.* The extended females are relatively inviable and are often sterile, owing to which peculiarities the form has been lost. Extended was never crossed to *melanogaster,* and no similar sex-linked type is known in that species.

Origin. Extended first appeared in a mass culture (Number 9,147) of 6 peach females mated to 8 forked dachs males. There were 4 extended females among the 107 offspring. Two of these females were mated to not-forked males (in separate cultures) and produced extended females, wild-type females, wild-type males, and forked males. The two types of males were separated in only one of these cultures, which gave 13 wild-type and 23 forked males. One may conclude that the original mutation occurred in one of the peach females of 9,147.

FACET (fa; I, 7.1)

Description. Facet is sexually dimorphic. The males have roughened eyes that are clearly and invariably distinguishable from those of wild-type. Facet females have somewhat roughened eyes, but are not usually sharply separable from wild-type, so that they cannot safely be used in collecting linkage data. Facet males mated to claret females give XO facet sons indistinguishable from the usual XY facet males, showing that the Y-chromosome is not responsible for the normal sexual dimorphism. The viability of facet is excellent, and both sexes are fully fertile. The character is recessive.

Comparison with Melanogaster. There are two sex-linked rough eyes in *melanogaster* not far from the locus of *simulans* facet—echinus and facet. Both these types were crossed to *simulans* facet. Echinus-facet hybrid females had smooth eyes, facet-facet hybrids had somewhat roughened eyes. It follows that facet is not allelomorphic to *melanogaster* echinus, and that it is recessive in hybrids (other matings show that *simulans* facet produces its characteristic effects in hybrid males). It is, however, not safe to conclude from these data that it is allelomorphic to *melanogaster* facet since hybrid females often have roughened eyes when neither parent had any rough-eyed mutant gene. It has been shown (Metz and Bridges, 1917, Mohr, 1923) that the notches of *melanogaster* are deficiencies for facet, and that notch-over-facet females show an exaggerated form both of notch and of facet. Accordingly, notch-11 females were mated to facet *simulans* males. There were produced 43 wild-type hybrid females, and 27 notch-facet hybrid females that were extreme in both characters. Other matings have shown that notch hybrid females (without facet) are not normally more extreme than notch *melanogaster.* One may conclude that facet *simulans* is allelomorphic to facet *melanogaster*—a conclusion that is in agreement with its locus and with its sexual dimorphism.

Origin. A female heterozygous for sepia, scarlet, and hairless (third chromosome genes) was mated to two sepia scarlet males (culture 19483). There were produced 237 offspring of the expected classes. There were seven facet males; the sex-ratio in the last few counts was 33 females to 50 males, showing that no sex-linked lethal was present. The mutation must have occurred in the oögonial tissue of the mother.

Two of these facet males were crossed, one to dusky forked females, the other to yellow white females. They gave the expected results (no non-disjunction). One wild-type daughter of the first male was tested, two daughters of the second one. All three gave the results to be expected from their parentage.

No further mutations at this locus have been detected in *simulans*.

FORKED (f; I, 56.0)

Description. Forked is very similar to the well-known character of the same name in *melanogaster,* though a little more extreme. It has been compared in detail with numerous similar characters in several species by Metz, Moses and Mason (1923). It is definitely less extreme than singed *simulans,* but more extreme than singed-2. Forked has excellent viability, and both sexes are fully fertile. It does not interfere with the classification of any other characters—except (presumably) the singed allelomorphs, with which it has not been tried.

Allelomorphism and origin. As previously stated (Sturtevant, 1921*a*), tests have shown that *simulans* forked and *melanogaster* forked are both recessive in hybrid females, and forked *melanogaster* by forked *simulans* gives forked hybrid females. It has also been shown that *simulans* forked produces its usual effects in hybrids of either sex that have no not-forked X present. The proof that forked *simulans* is allelomorphic to forked *melanogaster* is thus complete.

In the earlier account it was stated that Dr. C. B. Bridges found the original forked flies in a yellow stock. Dr. Bridges has checked up his notes for me, and it appears that at least two forked males were found in a wild-type (not yellow) stock of pure Florida ancestry. These males were mated to yellow females, and produced, as expected, wild-type daughters and yellow sons. Four of the wild-type females were tested, two of them by mating to the original forked males; all of these gave the results expected from $\frac{y}{f}$ females.

No further mutations have been detected in the forked locus of *simulans*.

FRAYED (II)

Description. This character has already been recorded (Sturtevant 1921*b*). The marginal hairs of the wing are irregular; some of the mesonotal hairs and bristles are missing; the remaining bristles are slightly reduced in size; the eyes are usually slightly roughened; the flies develop slowly (*i. e.,* emerge late), and are small, pale, and often sterile. The viability is poor. The character is recessive. It has been discarded. As reported, linkage counts gave 184/402 = 45.8 per cent as the separation frequency for frayed plum. The gene is presumably in the left half of the chromosome, but in the absence of other tests can not now be accurately placed on the map.

Origin. A few frayed flies were found, November, 1919, in a culture of pure Florida ancestry that formed part of the prune garnet linkage experiments.

FUSED (fu; I, 59.4)

Description. Fused resembles the character of the same name in *melanogaster* that has been figured by Morgan, Bridges, and Sturtevant (1925, p. 56), but the venation differs from wild-type rather less than does that of *melanogaster* fused. The most striking character is the approximation of the apices of the third and fourth longitudinal veins, usually accompanied by a thickening of the marginal vein in this region. The wings are usually held out at an angle from the longitudinal axis of the fly. The sternopleural bristles are usually doubled, and the ocellar bristles are always absent. The ocelli are usually absent, and the front (*i. e.,* the space on the head between the eyes, above the antennæ, and below the vertex) is broader than in wild-type, slightly concave, and quite bare. The viability of fused is somewhat lower than that of normal, and crossveinless greatly exaggerates this property, so that crossveinless fused is rarely produced at all (though there is no such relation between crossveinless and fused in *melanogaster*). Fused males are quite fertile; but the females, as in *melanogaster* (Lynch 1919), are often sterile and when they do breed do not produce fused offspring. Fused is completely recessive. It has been discarded, and the probable recurrence described below has not been kept.

Comparison with Melanogaster. As stated above, the fused of *melanogaster* is similar to that of *simulans*. *Melanogaster* females heterozygous for fused were mated to fused *simulans* males; there resulted 17 not-fused and 21 fused hybrid females. This shows that *simulans* fused is recessive in hybrid females and is allelomorphic to *melanogaster* fused.

Origin. On January 27, 1924, a single mosaic male was found in the semi-purple stock, largely descended from material collected at Kushla, Ala. The right wing of this male was fused, the left wing was wild-type. Mated to garnet forked females, he produced wild-type daughters and garnet forked sons. Four of these wild-type daughters were tested: one was +/g f, three were fu/g f. Evidently the mutation occurred in the early cleavage of the mosaic male, and both soma and germ-cells received both mutated and unmutated nuclei. A similar result is recorded for dusky in this paper.

In December, 1927, two different but closely related cultures (both small mass cultures) were found to contain numerous males that looked like fused. Four small mass cultures made up by mating these males to unrelated females all failed to produce offspring. The new type was evidently a sex-linked recessive, sterile in the males. It was not further studied, but the close resemblance to fused makes it very probable that the new character was allelomorphic to fused. It may be called fused-2.

GARNET (g; I, 42.3)

Description. This type was formerly described (Sturtevant, 1921*a*) under the name of carmine. Garnet is a pinkish eye-color, closely similar to ruby *simulans,* and to ruby, garnet, and pink *melanogaster.* It is rather darker than peach or claret *simulans* or peach *melanogaster.* The fertility and viability of garnet are very good. The character is recessive. Ruby garnet is not distinguishable from either single mutant type; garnet scarlet is yellowish in color, not different from scarlet peach or scarlet claret. Garnet has not been tried with any other eye-color types in *simulans.*

Comparison with Melanogaster. As previously reported, *melanogaster* garnet and *simulans* garnet are both recessive in hybrid females, and *simulans* garnet produces its usual effects in hybrid males and also in hybrid females that carry two garnet-bearing *simulans* X-chromosomes. It may be added that scarlet peach *melanogaster* by garnet *simulans* gives wild-type hybrid females. Since garnet *melanogaster* by garnet *simulans* gives garnet hybrid females, it follows that the two mutant genes are allelomorphic.

Origin. As previously reported, garnet first appeared as a single male that was produced by a small mass culture of pure Florida ancestry. The mother of the mutant male was heterozygous for yellow, and he was himself yellow.

HAIRLESS (H; III, 61)

Description. The fifth vein fails to reach the margin of the wing, and the fourth is also sometimes short. The mesonotal hairs are short, and many of the bristles are missing from the posterior borders of the abdominal segments. Many cephalic and thoracic bristles are missing, scars being usually present in the places where such bristles should occur. Counts, made on 6 specimens, indicate that the following bristles are affected: always absent—proclinate orbitals, convergent verticals, postverticals, presuturals: often absent—reclinate orbitals, divergent verticals, ocellars, sternopleurals, humerals, supra-alars, post-alars, dorsocentrals, scutellars. Polychæte hairless does not differ markedly from hairless in number of cephalic and thoracic bristles—not nearly as much as polychæte differs from wild-type. Diminished, on the other hand, has little effect on the bristle-number of not-hairless, but removes about half of the bristles of hairless—*i. e.,* hairless diminished has about half as many as hairless. Delta hairless has rather more bristles than hairless, and also has more normal venation than delta.

Hairless is dominant, and lethal when homozygous. The heterozygotes are of excellent viability and fertility, and are always easily separable from wild-type.

Comparison with Melanogaster. Hairless is dominant in *melanogaster* hybrids. As previously recorded (Sturtevant and Plunkett, 1926), and as described in this paper under Delta, tests have shown that it is allelomorphic to hairless *melanogaster.*

Origin. Hairless was found, April, 1924, as approximately half the offspring of a mating of a female $\frac{+}{\mathrm{y\ rb\ cv}}$ × four males y rb cv. One parent was evidently heterozygous for hairless, but it can not now be determined whether the character was not present in that specimen, or was not noticed when the mating was made. The strain traced to wild stocks from Lakeland, Florida; and Morristown, New Jersey, and to the ruby dusky stock.

INTERSEX (ix; II)

Description. This form has been described in detail (Sturtevant 1920*a*, 1921*b*). Both sexes are sterile and often have the wings extended. The males are not otherwise affected; but the females are partially changed into males in appearance. There are no sex-combs, seven abdominal tergites are present anterior to the genital region, there is a pair of chitinized spermathecæ, and a pair of small somewhat abnormally shaped egg-guides are present—these all being female characters. Male characters are an abnormally shaped "first genital tergite" with claspers, lateral anal plates, and dark tip to abdomen. The gonads are rudimentary, consisting almost entirely of tracheæ. The viability of intersex is good; but owing to its sterility and to the impossibility of classifying the males the character was difficult to keep, and has been lost. It was completely recessive. Its locus was not determined, though it was shown to cross over freely with plum.

Origin. Many intersexes were found (January, 1920) in a wild stock from Rochester, Minnesota.

KIDNEY-LIKE (II, III)

As previously recorded (Sturtevant 1921*b*), this is an irregularly appearing character dependent on genes in chromosome-II and in chromosome-III. The eyes are somewhat irregular in shape, usually emarginate anteriorly, the males are often sterile, and the females usually produce few offspring. The character is difficult to classify, and has been discarded. It was found, (December, 1919) in a culture of pure Florida ancestry that formed part of the yellow prune linkage experiments. Tests showed that kidney-like appears only in flies homozygous for a second-chromosome gene, and that most of the kidney-like flies were also homozygous for a third-chromosome "intensifier."

LETHAL–I (I)

Several sex-linked lethals have been encountered, but only one has been studied in any detail. That one proved to be a semi-lethal, and is here recorded under the name dwarf (*q.v.*).

LETHAL–II (II)

The only second-chromosome lethals studied are listed under the names arc and polychæte-lethal (*q.v.*). Semi-forked is also a semi-lethal.

LETHAL–IIIa (lIIIa)

Several matings of hairless ♀ × hairless ♂ were made from the original culture in which hairless appeared. Most of these gave approximately two hairless to one not-hairless offspring; but one mating gave (April, 1924) 202 hairless to 3 not-hairless. Matings of hairless × hairless were continued in this strain, and for the most part gave similar lethal ratios, though occasional cultures (evidently due to crossing over) gave approximations to the 2 : 1 ratio. The totals, for all matings of hairless × hairless among the uncrossed descendants of the original hairless culture are: from cultures giving approximately 2 : 1, 720 hairless to 326 not-hairless (a ratio of 2.2 to 1, indicating that hairless is at least as viable as wild-type); from cultures giving the lethal ratio, 1,115 hairless to 37 not-hairless. Calculations from this ratio indicate a crossover value of 6.4 per cent for hairless and the lethal.

Males of the constitution H/lIIIa were mated to delta females, and the delta offspring (Δ/lIIIa) were mated together. There were produced 484 delta, 39 wild-type. The indicated percentage of crossing over for Δ and lIIIa is 14.9.

The lethal may therefore be mapped 6.4 units to the left of hairless. It has been discarded.

MINUTE (M; III, 134)

Description. The bristles are somewhat shorter and finer than those of wild-type, but much less so than those of diminished or minute-IV. The minute flies also emerge rather later than wild-type (*i. e.*, their development period is longer). The character is rather variable, and sometimes overlaps wild-type. This is especially true in crosses to the scarlet peach stock, which evidently carries one or more modifiers of the minute character. Minute is dominant; homozygous minutes die.

Comparison with Melanogaster. The character resembles many *melanogaster* types, but its relation to them has not been studied. Minute is dominant in hybrids, its lethal effect being recessive as it is in pure *simulans*.

Origin. One minute male was produced (August 29, 1922) by a mating of $\frac{dy}{y\ pn}$ ♀ × y pn ♂, the ancestry tracing to yellow prune, ruby dusky, and Woods Hole wild stocks.

MINUTE-2 (M-2; II, 111±)

Description. This is a typical example of the minute class of mutants. The bristles are short and fine, the eyes slightly roughened, the wings often have small nicks in the margins, the flies are poorly viable, hatch late, are frequently sterile, and the females never produce large numbers of offspring. Minute-2 is dominant, like the typical minutes of *melanogaster.* Owing to its high degree of sterility, it has been lost.

Origin. A female $\frac{y\ w}{fa}$ was mated to four yellow white brothers. On February 1, 1926, a single minute-2 male was produced, there being no minutes among the 106 other offspring. This male was mated to $\frac{y\ w}{fa}$ sisters, and produced 10 minutes among 30 daughters, and 6 among 24 sons. Only one of these minutes produced offspring—a facet minute male that was mated to unrelated scarlet peach females and produced 20 minutes (7 females, 13 males) and 59 wild-type. One male from this culture was mated to scarlet peach females, and produced 11 minute, 5 minute scarlet peach, 46 wild-type, and 38 scarlet peach—showing the minute not to be in chromosome-III. A second male was mated to spread plum females, and produced 46 minute, 96 wild-type. Minute males from this culture mated to dash black polychæte spread plum females gave 58 minute, 167 spread plum, showing the minute to be in chromosome-II. Minute females from this last mating, mated to dash black polychæte spread plum males, gave 12 offspring; 1 M, 1 M py, 1 pm, 2 sd pm, 1 py sd pm, 1 b py sd pm, 1 dh, 4 dh b py sd pm. From these few data the most probable position for M-2 is to the right of pm, the crossover value being $\frac{1}{12}$ = 8 per cent.

MINUTE-4 (M-4; IV)

Description. Minute-4 is really the third minute studied in *simulans;* it is arbitrarily numbered 4 because it is in chromosome-IV, and because it corresponds to the minute-4 of *melanogaster* (see below). The bristles are small and fine, as the term "minute" always signifies in *Drosophila.* This is a relatively "slight" minute; the viability and fertility of both sexes is good, the development period is only slightly longer than that of wild-type, and the visible somatic effects, while always clearly discernible, are distinctly less extreme than those of minute-2 or of haplo-IV. The race has been lost.

Comparison with Melanogaster. Minute-4 resembles the minute-4 of *melanogaster.* Bridges has shown that the latter acts as an allelomorph of (deficiency for?) the fourth chromosome recessive rotated abdomen (received from Prof. S. S. Chetverikov). Four *melanogaster* females heterozygous for eyeless and for rotated abdomen were mated to six *simulans* males heterozygous for minute-4. There resulted 135 hybrid females: 88 wild-type, 35 minute, and 12 minute rotated abdomen. It follows that *simulans* minute-4, like *melanogaster* minute-4, is allelomorphic to rotated abdomen but not to eyeless.

Origin. On November 18, 1927, a single minute-4 female was found in culture 24364. The parents were a female heterozygous for yellow, white, and scarlet, and three males from the dusky forked stock. The original minute-4 female was heterozygous for scarlet, and for dusky and forked, but not for yellow and white.

The linkage data for minute-4 are discussed in this paper, under the heading "Independent inheritance of the four groups of genes."

NICK (vg^n, II, 77±)

Description. One or both wings show a small nick in the margin near the apex. The character overlaps normal; and errors in classification are also possible owing to the occasional occurrence of nicked wings due to other causes. The posterior scutellar bristles of nick are often erect, but this character is often slight or even absent. Although the viability and fertility of nick are good, its separability is so poor that it has been discarded. The character is recessive.

Comparison with Melanogaster. The erect scutellar bristles suggest the vestigial allelomorphs of *melanogaster,* and some of these allelomorphs have an effect on the wing scarcely more pronounced than that of *simulans* nick.

Accordingly, black purple vestigial arc speck *melanogaster* females were mated to black nick *simulans* males. There resulted 118 black hybrid females; nearly all of these had nicked wings, in some cases approaching such vestigial allelomorphs as strap or antlered in appearance. Most of these females also had erect posterior scutellar bristles. Vestigial has been shown to be recessive in hybrids. It may therefore be concluded that nick is allelomorphic to vestigial.

Origin. Twenty-seven nick flies were produced (January, 1926) by a culture that gave 105 not-nick flies. This culture was a back-cross linkage test for the third chromosome characters scarlet, delta, and aristapedia. Black and peach were also present in the strain.

PEACH (p; III, 106)

Description. Peach, as previously recorded, is an eye-color similar to ruby, garnet, and claret of *simulans,* and to peach, claret, ruby, garnet, etc., of *melanogaster.* It is rather paler in color than most of these types, but is much like *melanogaster* peach and *simulans* claret, the two palest of the series. Like all such colors, peach darkens with age, becoming somewhat brownish. Scarlet peach is a pale yellowish color. Peach is of excellent viability and fertility, and is always easily separable from wild-type.

Comparison with Melanogaster. Peach *simulans* has been crossed to the following *melanogaster* types: white, eosin, cherry, ruby, vermilion, purple, sepia, scarlet, peach, glass, cardinal, claret. Peach *melanogaster* has been crossed to *simulans* ruby, garnet, plum, scarlet, and peach. All these crosses yielded hybrids with wild-type eyes, except peach *melanogaster* × peach *simulans,* which has regularly given peach hybrids. Scarlet peach hybrids have also been seen, and have the yellowish eye-color expected. *Simulans* peach is clearly allelomorphic to *melanogaster* peach.

Origin. Several peach flies were found in August, 1920, in a wild stock that was collected in May, 1920, at Kushla, Alabama.

PLUM (pm; II, 103)

Description. The eye-color of plum closely resembles that of brown *melanogaster.* It is purplish, darker than the purple of *melanogaster,* but has a whitish reflection. It darkens much with age. Plum is sharply separable from wild-type, of excellent viability, and is fully fertile. It is, for these reasons, the most satisfactory second chromosome mutant type. Plum is completely recessive.

Plum scarlet is yellowish in color, nearly as pale as scarlet peach or scarlet claret. Plum claret is not distinguishable from claret.

Comparison with Melanogaster. Plum is recessive in hybrids. It has been crossed to the following mutant eye-colors of *melanogaster:* purple, brown, purploid, safranin, pink-wing, cinnabar, claret, peach, maroon, scarlet, eosin, and ruby. All these matings gave hybrids with wild-type eyes.

Origin. As previously reported (Sturtevant 1921*b*), plum was first found (December, 1919) as a single male in a wild stock from Staten Island, New York.

POLYCHÆTE (py; II, 74)

Description. The eyes are roughened, the hairs on the marginal vein of the wing are erect, and there are extensive modifications in bristle number. Most of the large bristles on the posterior margins of the abdominal segments are missing; the oral bristles are increased in number; the proclinate orbital is often doubled; there is usually an extra reclinate orbital; one or both verticals are sometimes doubled; the ocellars are usually missing; an extra postvertical is often present; one or more of the sternopleural hairs is often enlarged and bristle-like; the presutural, both notopleurals, the anterior supra-alar, and both post-alars are at times doubled; the posterior supra-alar is often absent; there may be as many as six dorsocentrals or six scutellars on each side, and the number of these is rarely as low as two (the wild-type number). The character is recessive. The viability and fertility of both sexes is good, and owing to the numerous effects of the gene classification is always easy.

The interaction effects of polychæte have been studied only with hairless; the bristle number of polychæte hairless is somewhat higher than that of hairless, but relatively much less so than is polychæte higher than wild-type.

Comparison with Melanogaster. Polychæte is recessive in crosses to *melanogaster.* No mutant character similar to it is known in that species.

Origin. Polychæte appeared in May, 1923, as roughly a quarter of the offspring from a mating of a $\frac{+}{\text{y rb dy f}}$ ♀ × y rb dy f ♂ (back-cross linkage test for yellow, ruby, dusky, forked). The parents of this culture were sibs; two other matings of males and females from the same source gave no polychætes. The mutation must have occurred in a grandparent of the first polychæte specimens—or in a still earlier generation. The pedigree is complicated, tracing to stocks from St. Augustine, Florida; Lakeland, Florida, and other localities.

POLYCHÆTE-LETHAL (py-l; 74)

Origin. In September, 1924, a female of the constitution $\frac{\text{dh b py sd pm}}{\text{sw}}$ was mated to three males that were straw spread plum. Among the 189 offspring of the expected types was one polychæte spread female. There was no polychæte in the ancestry of the straw spread plum fathers, and no other suggestion has been found of the presence of a polychæte allelomorph in that strain. The exceptional polychæte spread female was tested; her offspring (several of which were themselves tested) showed her constitution to have been $\frac{\text{py sd}}{\text{sw py-l sd pm}}$. As indicated by its name, polychæte-lethal was found to be a completely lethal allelomorph of polychæte. Flies heterozygous both for polychæte and for polychæte-lethal are not different in appearance from homozygous polychæte. Wild-type heterozygous for polychæte-lethal is not distinguishable from wild-type. Polychæte-lethal has been discarded, as it was not useful enough to make it seem worth while to keep a selected stock.

PRUNE (pn; I, 2.9)

Description. This type has already been recorded (Sturtevant 1921*a*). The eyes are darker than those of wild-type, slightly purplish in tinge, and have a whitish rather than a black "fleck." The color closely resembles that of prune *melanogaster*. The viability and fertility of prune are very good. The character is recessive. It has not been used extensively in linkage experiments, as it could presumably not be used together with any other sex-linked eye-color, and white and ruby are more favorably placed for most experiments.

Comparison with Melanogaster. The following *melanogaster* types have been mated to prune *simulans:* notch-8, coral, eosin, ruby, and prune. All gave hybrid females with wild-type eyes, except the prune *melanogaster*, which gave prune hybrid females. *Simulans* prune is therefore allelomorphic to *melanogaster* prune. *Simulans* prune produces its usual effects in hybrid males.

Origin. As previously reported, prune first appeared in a single male from a small mass culture of pure Florida ancestry.

ROUGHISH (III)

Description. As previously reported (Sturtevant 1921*b*), this is a slightly roughened eye, not always completely separable from wild-type. It is recessive. Owing to the uncertainty of its classification, roughish was discarded before its locus was determined. As reported, it gave $\frac{516}{1177} = 43.8\%$ as a crossover value with scarlet. Other experiments indicate quite free crossing over with delta and with peach; but these data are too unreliable to publish, or to use in placing roughish on the map.

Comparison with Melanogaster. As recorded, roughish was crossed to the *melanogaster* types roughoid and rough, giving wild-type hybrids in both cases.

Origin. Roughish was found (February, 1920) widely scattered in a stock from Rochester, Minnesota, and in several outcrossed descendants of this stock.

ROUGHISH-B (rh-b; III, 26)

Description. This is a slightly roughened eye, rather more extreme than roughish, less so than rugose. It is usually separable from wild-type, though there is probably a small percentage of error. The character is recessive. It has been discarded.

Origin. Roughish-b was found (January, 1924) as several males and females in an F_2 count (from a small mass-culture of F_1 flies) from a cross of yellow (pure Florida ancestry) × wild-type of a stock from Austin, Texas.

RUBY (rb; I, 9.7)

Description. Ruby was first described (Sturtevant 1921*a*) under the name rubyoid. The color resembles ruby, garnet, or pink of *melanogaster*, or garnet of *simulans*. It is rather darker than peach of these species. Ruby garnet is not distinguishable from either single mutant type, ruby scarlet is yellowish in color; no other eye-colors have been tested with ruby. The viability of ruby is good, and both sexes are fully fertile. The character is recessive.

Comparison with Melanogaster. Scarlet peach *melanogaster* by ruby *simulans* gives wild-type hybrid females; ruby *melanogaster* × prune, garnet, plum, scarlet, or peach *simulans* also gives wild-type hybrid females. Ruby *melanogaster* × ruby *simulans* gives ruby hybrid females. These results show clearly that the two characters are allelomorphic.

Origin. As previously recorded, ruby first appeared (October, 1919) as a portion (presumably half) of the sons from a female that was $\frac{+}{\text{ypn}}$, the ruby sons being mostly not yellow. Their mother had received a ruby-bearing X instead of the expected wild-type one from her mother.

In July, 1922, two ruby males were found in a black stock (wholly descended from flies collected at Morristown, New Jersey). Both these males were black, and no ruby black had been seen before, from which it follows that this was a recurrence of the ruby mutation, not a case of contamination of the black stock. The new ruby was tested with the old one and found to be allelomorphic to it. Since it seemed not to differ at all from the old one, it was discarded.

RUDIMENTARY (r; I, 53.3)

Description. The wings of rudimentary are usually shorter than those of wild-type, often squared off at the apex, and almost always have the marginal hairs standing out at right angles to the marginal vein. The character overlaps wild-type at times and also has low viability. Both these properties make it unfavorable for linkage work, it being necessary in most cases to calculate the linkage values only from the flies that actually show the rudimentary character. The rudimentary females are also often infertile. Owing to these various undesirable characteristics, rudimentary has been discarded.

Comparison with Melanogaster. Rudimentary resembles some of the less extreme rudimentary allelomorphs of *melanogaster.* Accordingly *melanogaster* females that were $\frac{r^7}{\text{B}}$ were crossed to *simulans* rudimentary males. There resulted bar hybrid females and rudimentary hybrid females. One may conclude that the *simulans* type is allelomorphic to *melanogaster* rudimentary.

Origin. A single rudimentary male appeared December 10, 1920, in a small mass culture from dachs peach females mated to scarlet males. This male was mated to yellow forked females, and produced wild-type daughters and yellow forked sons. The three wild-type daughters that were tested all gave the results expected from $\frac{\text{y f}}{\text{r}}$ females.

No further mutations at this locus have been detected in *simulans.*

RUGOSE (rg; III, 109)

Description. The eyes of rugose are distinctly roughened, scarcely (if at all) smaller than those of wild-type, and without black "smudges." The type recalls echinus of *melanogaster*. It is sharply separable from wild-type, and of good viability and fertility. Rugose is recessive. The strain has, unfortunately, been lost.

Comparison with Melanogaster. Rugose was mated to roughoid and to rough *melanogaster*. Both crosses gave wild-type hybrids.

Origin. Many rugose flies were found, September, 1920, in a yellow forked stock of pure Florida ancestry.

SCARLET (st; III, 42)

Description. Scarlet is a bright red eye-color, closely resembling vermilion or scarlet of *melanogaster* (*see* Sturtevant 1921*b*). The ocelli are colorless. Scarlet prune, scarlet garnet, scarlet plum, scarlet peach, and scarlet claret, are all yellowish in color, and all look somewhat alike, their differences being rather less than are the differences between the corresponding types in the absence of scarlet. White scarlet is white; sepia scarlet is somewhat paler than sepia when it is young, but is satisfactorily separable from sepia only through examining the ocelli. Scarlet is recessive (though flies heterozygous for white, scarlet, and claret show a slightly duller color than wild-type). The viability and fertility of scarlet are excellent, and it can be classified accurately in combination with any other simulans character except white.

Comparison with Melanogaster. As already recorded, *melanogaster* scarlet and *simulans* scarlet are both recessive in the hybrids, while scarlet *melanogaster* × scarlet *simulans* gives scarlet hybrids. Scarlet peach hybrids have also been seen; they have the color expected. Scarlet *simulans* has been crossed to the following *melanogaster* types: white, eosin, ruby, vermilion, purple, peach, claret. In every case the resulting hybrids were wild-type in eye-color. Scarlet *melanogaster* has been crossed to ruby, garnet, plum, and peach *simulans*, again with the production only of wild-type eye-color. The case for allelomorphism of *melanogaster* scarlet and *simulans* scarlet may therefore be taken as fully proven.

Origin. As previously reported, scarlet appeared in two individuals in a mass culture of pure Florida ancestry (August, 1919). It was the first autosomal mutant type found in *simulans*.

SEMI-FORKED (II)

Description. The bristles are somewhat curved and are shorter and relatively thicker than those of wild-type. The flies are usually small, hatch rather later, and have a viability only about 20 per cent that of wild-type (see below). All those tested were sterile. The character, which was recessive, was discarded before its locus was accurately determined.

Origin. As described under the mutation arc, an arc-bearing second chromosome (from the peach stock, of pure Alabama ancestry) was kept opposite a spread plum chromosome by mating sd pm ♀ × $\frac{\text{ar}}{\text{sd pm}}$ ♂ in successive generations. When wild-type flies $\left(\frac{\text{ar}}{\text{sd pm}}\right)$ from this strain were mated together there resulted 514 wild-type (including some spreads), 318 plum (including some spread plum), and 36 semi-forked. This result is intelligible on the assumption that there were two lethals in the ar chromosome. One of these was presumably ar itself, the other was probably semi-forked acting as a semi-lethal. On this assumption the locus of semi-forked can be approximated. In general, if a mating is made of $\frac{l_1\,l_2}{a}$ ♀ × $\frac{l_1\,l_2}{a}$ ♂, where l_1 and l_2 are lethals and a is a recessive mutant gene, the following relations hold.[1] The percentage of aa offspring is independent of the locus of *a*, being determined solely by the amount of crossing over between l_1 and l_2. The amount of that crossing over is equal to $\frac{2a-A}{a}$, where A and a represent the numbers of individuals of the wild-type and mutant classes, respectively. Also, if l_1 is not completely lethal, its coefficient of viability may be calculated; for if it were not lethal at all it should appear in a number of individuals equal to A-a.

In the present instance, considering spread, A = 508, a = 324; considering plum, A = 514, a = 318. Taking the average of these, A = 511, a = 321. Substituting these values, semi-forked arc crossing over $= \frac{2\,(321) - 511}{321} = \frac{131}{321} = 41$ per cent, and semi-forked viability $= \frac{36}{511-321} = \frac{36}{190} = 19$ per cent.

SEMI-PURPLE (s-p; II, 53±)

Description. This is the "purplish" of Morgan, Bridges, and Sturtevant (1925). Semi-purple is a dark purplish eye-color, that rapidly approaches wild-type as the flies age. It is never strikingly different from wild-type, and classifications are always subject to some error. Accordingly, the character has been little used, though the viability and fertility of semi-purple are good. The character is recessive. Tests have not been made with other eye-colors. Some such combinations would perhaps give sharply separable classes.

Comparison with Melanogaster. Semi-purple has been crossed to the following *melanogaster* types: purple, purple cinnabar, safranin, purploid, brown-2. In all these cases wild-type hybrids were produced.

Origin and locus. Several specimens of semi-purple were found, August, 1923, in a stock collected at Kushla, Alabama, in 1921. Crosses of these to black gave no black semi-purple in F_2, and later experiments showed that semi-purple lies not far from one-third of the distance from black to polychæte; the data involve so much error in classification that they are not here recorded.

SEPIA (se; III, 7)

Description. Sepia is a dark brownish eye-color, closely resembling the sepia of *melanogaster*. When sepia first emerges it is yellowish red in color. As the fly ages it gradually darkens, passing through brownish stages until it ultimately becomes very dark blackish brown. It is at all times easily separable from wild-type, but in combination with other eye-colors it is not easy to classify. Sepia scarlet resembles sepia, though somewhat paler when young. This combination can, however, be used satisfactorily by taking advantage of the fact that scarlet makes the ocelli colorless while sepia does not do so. Sepia flies are of excellent viability and fertility. The character is recessive.

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[1] Provided no crossing over occurs in the male.

Comparison with Melanogaster. Melanogaster sepia and *simulans* sepia have both been shown to be recessive in hybrids, sepia *melanogaster* by sepia *simulans* gives sepia hybrids. *Simulans* sepia is therefore allelomorphic to *melanogaster* sepia.

Origin. Numerous sepia males and females were found, August, 1924, in a wild stock that was collected in 1922 at Woods Hole, Mass.

SHAVEN-ABDOMEN (S-a; II, 61)

Description. Some of the bristles normally present on the posterior margin of each abdominal segment are missing in this type. The character overlaps normal—usually in the majority of the specimens that are genetically shaven-abdomen. The character is dominant; the nature and viability of the homozygote were not determined. The fertility and viability of both sexes is good; but the separability is so poor that the character has been discarded.

Origin. Shaven-abdomen first appeared (January, 1922) in 35 of the 88 offspring produced by a small mass culture of peach females by black males. Tests of the shaven-abdomen males by black females gave all the shaven-abdomen offspring also black, showing that the mutant gene was present in one or more of the black males used in the original culture. These black males were descended entirely from a wild-stock collected at Morristown, N. J., in 1921.

SINGED (sn; I, 21.1)

Description. The bristles and hairs of singed are curved, often bent at sharp angles, somewhat shortened and thickened, and occasionally forked. These effects are especially obvious in the longer bristles, but the shortening and thickening, and often the curving, appear to be present in all the hairs and shorter bristles (except the branches of the arista and the minute hairs on the palpi and third antennal joint). The character is not noticeably variable, and is always sharply separable from wild-type. Its viability is good. Singed females are completely sterile, but the males breed freely.

Singed has not shown any special interaction effects with other types, but has not been studied with any other bristle character except polychæte. Singed polychæte shows both characters clearly, and apparently without modification.

Singed is completely recessive, apparently not producing the effect on the size of the posterior scutellar bristles described by Metz, Moses and Mason (1923) for the heterozygotes of similar characters in other species.

Comparison with other species. Singed closely resembles the singed and forked-5 types of *melanogaster.* Metz, Moses and Mason (1923) have given a detailed description of these and the other more or less similar types in *melanogaster, virilis, willistoni, funebris,* and *obscura.*

Simulans singed has been found to produce its usual effects in hybrid males, and singed-3 *melanogaster* ♀ × singed *simulans* ♂ has produced 57 hybrid females, all clearly singed. Although neither singed has actually been demonstrated to be recessive in hybrid females, it seems safe to conclude that they are allelomorphic.

Origin. Culture 14961 was made up by mating a single female, heterozygous for black and for polychæte, to 3 black polychæte spread males. Of the 283 offspring, 127 were not-polychæte, 155 were polychæte, and one was a gynandromorph with the female parts polychæte and the male parts not-polychæte—presumably a double-nucleus gynandromorph, since polychæte is a second-chromosome recessive. The sexes were not separated in the early counts from 14961, but were equal in number (41 ♀ : 44 ♂) in the later counts. Singed appeared as a single polychæte male in this culture. The mutation presumably occurred late in the germ-track of his mother.

The original singed male was mated to yellow females, and produced wild-type daughters and yellow sons. Three of the wild-type daughters were tested, and all proved to be heterozygous both for yellow and for singed.

SINGED-2 (sn^2; I, 21.1)

Description. Singed-2 is less extreme than singed, and is sexually dimorphic. The females, though sterile as in singed, show the character slightly and are sometimes difficult to distinguish from wild-type. The males are never difficult to classify, but are less extreme than are singed males. Singed-2 males have been mated to claret females; the resulting XO singed-2 males were not distinguishable from XY specimens. It follows that the Y chromosome is not responsible for the sexual dimorphism of the type. The singed-2 race has been discarded.

Allelomorphism. Singed-2 was not tested in *melanogaster* hybrids, but $sn^2/+$ ♀ × sn ♂ (both *simulans*) gave 32 wild-type females and 25 females intermediate between sn and sn^2 in appearance. Hence these characters depend on allelomorphic genes.

Origin. Culture 21094 was made up by mating a female $\frac{\text{fa rb dy f}}{\text{y}}$ × two males y fa rb. There resulted 106 females and 109 males of the expected classes. One of the facet ruby males (*i. e.,* a rb-dy crossover) was the original singed-2 specimen. The mutation must have occurred in the mother, and it is possibly significant that the mutant egg underwent crossing over between ruby and dusky—within which region the singed locus lies.

The original mutant male was mated to yellow females, and produced wild-type daughters and yellow sons. Eight of the wild-type daughters were tested, and all gave the results expected from $\frac{\text{fa rb sn}^2}{\text{y}}$ females.

SPREAD (sd; II, 80)

Description. The wings of spread are held out at an angle from the long axis of the body which varies approximately between 30° and 90°. The wings are held flat as in wild-type. There is no other peculiarity of the type, which is sometimes difficult to classify—since similar extended wings at times appear in almost any stock of *Drosophila*. The error in classification is in most cases, however, negligibly small—though in dead specimens or in those whose wings have not expanded classification is not possible. The character is recessive. Its viability and fertility are good.

Comparison with Melanogaster. Spread is recessive in hybrids, as in pure *simulans.* It has been crossed to curved *melanogaster,* with the production of wild-type hybrids.

Origin. As previously recorded (Sturtevant 1921*b*), spread was found first as a number of flies of both sexes in a yellow-prune stock, of mixed Florida and Cold Spring Harbor (New York) ancestry.

STRAW (sw; II, 61)

Description. Straw is a pale body color, slightly more yellowish than wild-type but much less extreme than yellow. The bristles are more yellow than those of wild-type, and this is the most convenient way of detecting the character. At best, straw is difficult to classify, but with experience it is possible to identify it in most specimens. In cultures segregating both for black and for straw the difficulty of classification is still greater; but even here it is possible to identify most specimens accurately. Straw is recessive. Its viability and fertility are good, but it has not been used extensively because of the difficulty of classification.

Comparison with Melanogaster. The straw type in *melanogaster* is even less distinct from wild-type than is that of *simulans,* and for this reason its position on the second chromosome map has not been determined. Straw *melanogaster* mated to straw *simulans* has given hybrids that appeared to be straw; but this result needs verification owing to the nearness of straw to wild-type in appearance. The case has not been studied further because the allelomorphism does not help the *simulans* map, the locus of *melanogaster* straw being unknown.

Origin. Straw appeared, July, 1924, as approximately 25% of the offspring of a pair of hairless flies that formed a part of a selection experiment designed to decrease the number of bristles on hairless flies. The strain had been inbred, brother to sister, for six successive generations beginning with the culture in which hairless first appeared. The mutation to straw probably occurred during this process of inbreeding. The strain traced to wild stocks from Lakeland, Fla., Morristown, N. J., and other places.

TINY-BRISTLE-A (tb-a; I, 56.6)

Description. As previously reported (Sturtevant 1921*a*), this type has small bristles, somewhat roughened eyes, and rather faint and irregular abdominal bands. The development period is longer than that of normal, the viability is often low, and the females are sterile. So far as tested, the fertility of the males appears to be normal. The character is recessive. No unusual interaction effects have been seen, but tiny-bristle-a has not been tried with any other bristle character except forked. Forked tiny-bristle-a shows both characters—the bristles are small and of the usual forked form. Tiny-bristle-a has been discarded.

Comparison with Melanogaster. Tiny-bristle-a resembles a number of *melanogaster* types, but is most like the tiny-bristle of that species. As previously reported, however, the two types are not allelomorphic, since hybrid females, heterozygous for both, are wild-type in appearance.

Origin. As reported, tiny-bristle-a was first detected in half (38 in a total of 66) the sons of a single female from a wild stock collected in 1919 at Lakeland, Florida.

TINY-BRISTLE-B (tb-b; I, 42.7)

Description. This character is less extreme than tiny-bristle-a. The bristles are short, though not so fine as in tiny-bristle-a. No other effects have been observed: the abdomen and eyes are normal, the development period is not noticeably lengthened, and both sexes are fully fertile. The character is recessive. It has been lost.

Comparison with Melanogaster. Tiny-bristle-b was not crossed to *melanogaster,* as there did not appear to be any probable allelomorphs of it in that species, at the time tiny-bristle-b was in existence.

Origin. Tiny-bristle-b was found in August, 1920, in several males from a small mass-culture. The mothers of this culture were all either homozygous or heterozygous for scarlet, and were of mixed Florida and Minnesota ancestry.

TRUNCATE (T; II, 0)

Description. The wings are blunt at the apex, often markedly so, sometimes with a concave margin instead of the normal convex one. The character is quite variable, even the two wings of a given specimen often differing. It overlaps wild-type frequently. In some cases (see below) "vortex" is present—*i. e.,* the hairs on the dorsal surface of the thorax, lateral to the dorsocentral rows, are somewhat whorled. This may occur on one or both sides of the thorax. In extreme cases there is a chitinous papilla at the center of the whorl.

Truncate is dominant. The viability and fertility of the heterozygotes are good, but the usefulness of the character is seriously limited by the frequency with which it overlaps normal. The homozygous truncates are probably not viable. No rigorous test has been made, but long-continued selection of extreme truncates has not resulted in producing any cultures with more than two-thirds of the offspring truncate. The homozygotes, if they ever survive, must be sterile.

Comparison with Melanogaster. Truncate resembles several of the truncate series of allelomorphs in *melanogaster,* in which both the truncated wing-shape and the vortices on the thorax occur. Dumpy, a recessive showing both these characters, has been used in the tests. Preliminary tests showed that *simulans* truncate is dominant in the hybrids at least to the same extent as in pure *simulans,* and that dumpy is recessive in the hybrids as it is in *melanogaster.* Dumpy × truncate gave 9 wild-type hybrid females. Dumpy black *melanogaster* females × T/b *simulans* males gave 15 black hybrid females, and one not-black hybrid female that was extreme vortex, wings mere pads with blunt tips, and legs much shortened. Evidently $\frac{\mathrm{T}}{\mathrm{dp}}$ hybrids are poorly viable and have the truncate characters developed in more extreme form than is ever produced by either mutation itself. One may conclude that truncate *simulans* belongs to the truncate-dumpy series of *melanogaster*.

Origin. Truncate first appeared, March 17, 1926, as a single male from a mating of bobbed females by sepia males. The sepia males, which carried a Y-chromosome without a bobbed allelomorph, were descended entirely from a wild stock collected at Woods Hole, Mass., in 1922. The pedigree of the bobbed females is very complex, but goes chiefly to the dusky forked stock and to the culture that gave rise to the second bobbed mutation.

The original mutant male showed the vortex character, and was not observed to have modified wings. Mated to sepia scarlet females, he gave offspring of both sexes, both among the sepias and among the not-sepias, that had wings clearly truncated. The vortex character did not reappear for several generations, and has been found only in occasional specimens, except in one strain descended from the original male. In this strain the vortex character is more frequent and more pronounced than the truncate character. The genetic basis of this difference has not yet been determined—it may be that modifying genes are responsible, or it may be that a different allelomorph of truncate is present, since several different allelomorphs are known in *melanogaster*.

VESICULATED (vs; I, 16.4)

Description. One or both wings are somewhat crumpled, often (especially when newly expanded) with small vesicles filled with fluid. In older flies the wings are usually somewhat opaque. The character is not favorable for linkage work, as it often overlaps wild-type. Crossover values can be calculated only on the basis of those specimens that actually show the vesiculated character. This variability is due, in part at least, to modifying genes. An unselected pure stock of vesiculated came to look quite like wild-type, but outcrosses led to the recovery of typical vesiculated specimens, showing that the character had disappeared through the piling up of modifiers and not through a change in the vesiculated gene itself. Both sexes are fully fertile. Vesiculated is recessive.

Comparison with Melanogaster. Vesiculated resembles the character of the same name in *melanogaster* described by Evang (1925), though *melanogaster* vesiculated overlaps wild-type more rarely—if at all. A cross of vesiculated *melanogaster* by vesiculated *simulans* gave 38 hybrid females, of which 36 were clearly vesiculated. Evidently the two are allelomorphic.

Origin. Vesiculated appeared in December, 1920. Three females $\frac{E\ f}{+}$ (of Alabama and Florida ancestry) were mated to yellow forked males. There were produced 12 extended females, 19 not-extended females, 12 vesiculated males, 6 wild-type males, and 4 forked males. Evidently vesiculated was present in the wild-type (paternal) X-chromosome of at least one of the extended females (probably only one of the three females used produced offspring).

WHITE (w; I, 4.1)

Description. The eyes of the white-eyed type are pure white, the ocelli are colorless, and the testicular envelop is whitish. All these characters agree perfectly with those of the white of *melanogaster*. The eye-color is always easily and completely separable from any other color known in *simulans,* and is not at all variable. The viability of white is very good, and both sexes are of normal fertility.

All other eye-colors tested with white have given the same result as in *melanogaster*—the double mutant types are indistinguishable from simple white. White is recessive, but a slightly duller and more brownish color than that of wild-type has been observed in flies heterozygous for white, scarlet, and claret simultaneously.

Comparison with Melanogaster. As stated, white is closely similar to the white of *melanogaster.* Both these types have been found to be recessive in the *melanogaster-simulans* hybrids, as have the white allelomorphs coral, cherry, eosin and apricot of *melanogaster.* It has also been found that *simulans* white produces its normal effects in hybrid males. *Simulans* white mated to *melanogaster* white has given white hybrid females, and *simulans* white mated to *melanogaster* ivory (a pale allelomorph of white) has given hybrid females with a faint tinge of eye-color. It may therefore be taken as established that the white of *simulans* is allelomorphic to that of *melanogaster.*

Origin. On April 26, 1924, a number of white males were found in a wild-stock that had been collected at Morristown, N. J., in 1921. Such males were found in this stock almost every time it was examined for several months afterwards. No analysis is possible, as the mutation had probably occurred several generations before the first specimens were discovered.

I have not detected any other mutations at this locus in *simulans,* but Dr. H. Nachtsheim (unpublished data) has studied a recurrence of the white mutation.

YELLOW (y; I, 0)

Description. The hairs and bristles are yellowish brown, instead of the black or very dark brown characteristic of wild-type (Sturtevant 1921*a*). Yellow is always easily separable from wild-type. Its viability is usually good, but is sometimes noticeably low in combinations with other mutant types (especially with dusky). Both sexes are fully fertile. The character is recessive. Yellow does not interfere with the classification of any other sex-linked character. It has not been tried with straw; with black it gives a brownish color, easily distinguishable from black or from wild-type, but somewhat less distinct from yellow than black is from wild-type.

Comparison with Melanogaster. Yellow is closely similar to the yellow of *melanogaster.* Each of these is recessive in hybrid females, and *simulans* yellow produces its usual effects in hybrid males and in hybrid females with two yellow-bearing *simulans* X-chromosomes. Yellow *melanogaster* by yellow *simulans* gives yellow hybrid females. The two are therefore allelomorphic, as previously reported.

Origin. Dr. C. W. Metz found a wild yellow male at Lakeland, Fla., in 1919; all the linkage experiments concerning yellow have been with this strain. As previously reported (Sturtevant 1921*a*), a single yellow male was found (1919) in a wild stock from New Hampshire, and this was found to be not distinguishable from the earlier yellow, and allelomorphic to it.

There was, however, no proof that this was a separate mutation, rather than a case of contamination.

More recently a single yellow male was found in a culture containing the mutant polychæte. The yellow male was polychæte, thus showing him not to be due to contamination. He was sterile, so that it is not certain that this represents another mutation at the yellow locus.

No other mutations at this locus have been detected in *simulans*.

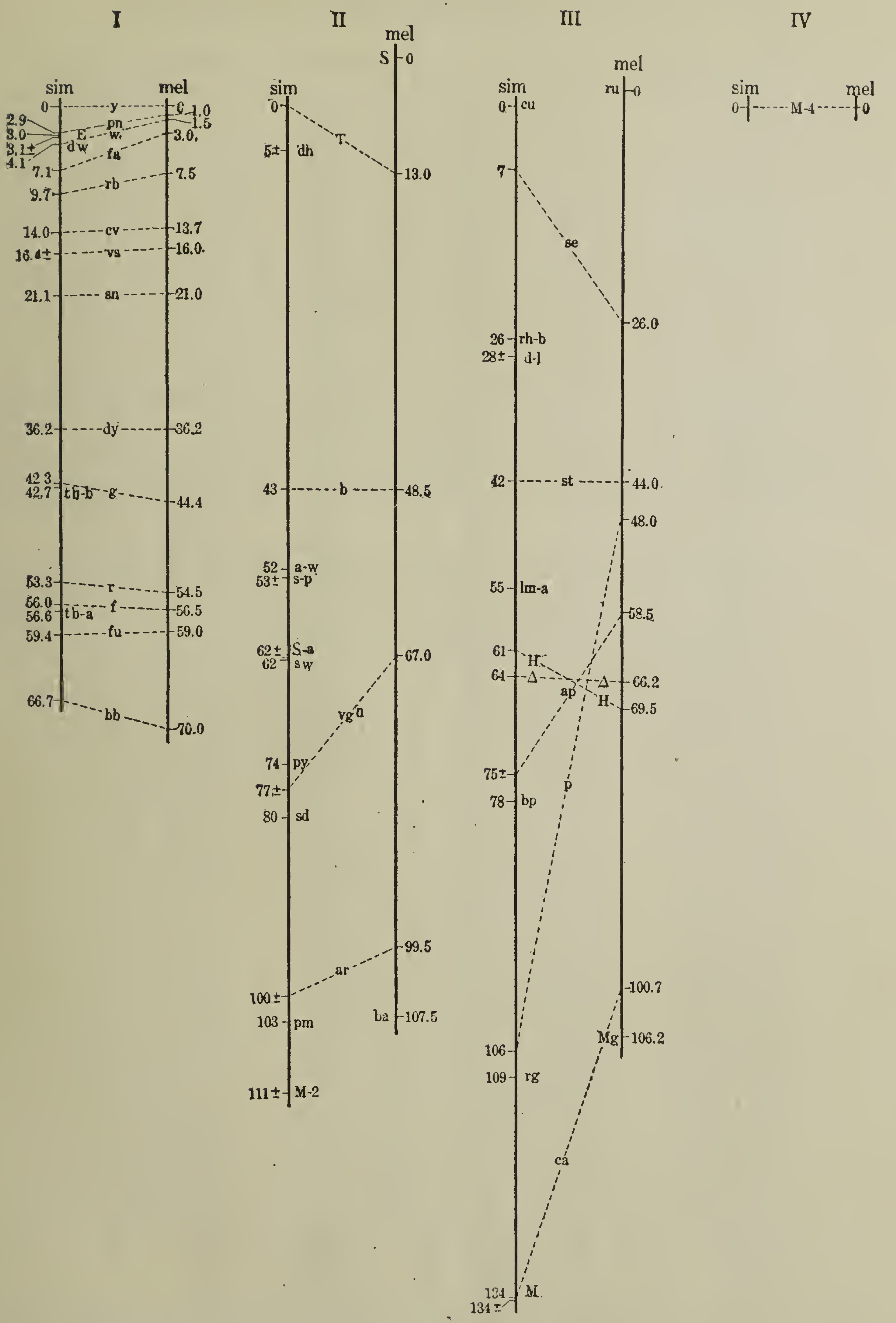
MAPS OF THE *SIMULANS* LINKAGE GROUPS

The linkage data of tables 4 to 6 (themselves summaries of tables 12 to 22 and of all previously published data), are summarized in the maps of figure 1. These were constructed in the following way. The experiments of the tables that include more than two loci at a time establish the sequence of most of the loci; in a few cases there is some uncertainty, and such loci are entered on the map with a "±" sign after their numbers. Vesiculated (vs), for example, is given as 16.4±; this means not only that the value 16.4 is an approximation, but also that it was not directly proven that vs lies to the right of cv, the nearest locus to it. This significance is to be attached to the "±" sign in all cases except that of dash (dh) in chromosome-II; in this instance the sequence is certain, but the distance from T (the nearest locus) may be much greater or less than is shown on the map.

Each map is based primarily on the values for successive sections. The loci chosen were: in chromosome-I: y, rb, sn, dy, g, f, bb; in II: T, b, py, sd, pm; in III: se, rh-b, st, H, Δ, bp, p, M. The crossover values observed for the successive intervals between these loci were used directly, in no case making corrections for unobservable double-crossing-over within a section (from which it follows that the maps of II and III, at least, are likely to be lengthened rather than shortened when more loci are studied). The remaining loci were then entered on the map by means of their crossover values with the nearest "standard" locus with which data were available.

The *melanogaster* maps given for comparison are slightly modified from those published by Morgan, Sturtevant, and Bridges (1925). The rightmost and leftmost known locus in each chromosome is shown, and also all loci known to correspond to *simulans* loci—these latter being connected by dotted lines.

A study of these maps shows that there has been no case detected in which genes have been shifted from one chromosome to another as between the two species. In chromosome-I the two are alike as to sequence. It should be pointed out also that the spindle fiber is known to be attached at the bobbed end in both species. There is distinctly more crossing over near the "left" (or "upper") end of the *simulans* chromosome; otherwise, even the crossover values are not noticeably different in the two species. Chromosome-II is not well understood in *simulans*. The four corresponding loci are in the same sequence, but the crossover values are sufficiently different to make a more detailed comparison desirable. The *simulans* chromosome-III map is the longest one yet recorded except that of the X of *D. obscura* (Lancefield 1922), and as pointed out above, further study is likely to lengthen it still more. The section including H, Δ, ap, and p is clearly in-


I
sim
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0
y
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1.0
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E
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7.5
rb
9.7
14.0
cv
13.7
16.4±
vs
16.0
21.1
sn
21.0
36.2
dy
36.2
42.3
42.7
tb-b
g
44.4
53.3
r
54.5
56.0
56.6
tb-a
f
56.5
59.4
fu
59.0
66.7
bb
70.0
II
sim
mel
S
0
0
T
5±
dh
13.0
43
b
48.5
52
a-w
53±
s-p
62±
S-a
62
sw
67.0
vg
74
py
77±
80
sd
99.5
ar
100±
103
pm
ba
107.5
111±
M-2
III
sim
mel
ru
0
0
cu
7
se
26.0
26
rh-b
28±
d-l
42
st
44.0
48.0
55
lm-a
58.5
61
H
64
Δ
ap
Δ
66.2
H
69.5
75±
78
bp
p
100.7
Mg
106.2
106
109
rg
ca
134
M
134±
IV
sim
mel
0
M-4
0

FIG. 1.

TABLE 4—*Summary of all linkage data on X-chromosome*

Loci	Total flies	Crossovers	Per cent
y pn	7,960	230	2.9
y E	888	32	3.6
y w	8,438	345	4.1
y fa	6,530	324	5.0
y rb	14,779	1,436	9.7
y cv	5,623	784	14.0
y vs	334	72	21.6
y sn	5,200	984	18.9
y dy	17,320	6,060	35.0
y g	15,997	5,844	36.5
y tb-b	1,275	436	34.3
y r	425	179	42.2
y f	24,232	10,665	44.0
y tb	6,977	3,194	45.8
y fu	2,835	1,309	46.3
y bb	986	478	48.5
pn E	708	1	0.1
pn fa	310	13	4.2
pn dy	1,392	546	39.3
pn tb-b	232	93	40.2
pn f	1,010	432	42.8
pn tb	973	406	41.7
E rb	383	31	8.1
w fa	3,230	63	2.0
w rb	345	23	6.7
w cv	345	45	13.0
w vs	79	6	7.6
w sn	1,154	244	21.1
w dy	5,185	1,845	35.6
w f	4,747	2,117	44.6
w fu	837	363	43.4
w bb	320	155	48.4
fa rb	3,451	89	2.6
fa sn	1,521	170	11.2
fa dy	4,615	1,359	29.5
fa f	3,275	1,404	42.8
rb cv	5,934	257	4.3
rb vs	210	20	9.5
rb sn	4,243	485	11.4
rb dy	9,750	2,405	24.8
rb tb-b	962	246	25.6
rb f	6,811	2,632	38.5
rb tb	1,126	424	37.6
rb fu	555	218	39.3
rb bb	548	235	42.8
cv sn	2,937	211	7.2
cv dy	3,801	761	20.1
cv g	1,505	425	28.3
cv f	1,870	723	38.7
vs sn	79	5	6.3
vs g	333	76	22.8
vs f	641	208	32.4
sn dy	3,854	566	14.7
sn g	1,068	242	22.6
dy g	8,893	543	6.1
dy f	18,798	4,085	21.7
dy fu	1,528	361	23.6
dy bb	1,455	448	30.4
g tb-b	758	3	0.4
g r	408	37	9.1

TABLE 4—Continued

Loci	Total flies	Crossovers	Per cent
g f	16,145	2,228	13.7
g tb	3,311	492	14.9
g fu	1,704	311	18.3
g bb	770	163	21.9
tb-b f	149	19	12.8
r f	518	14	2.7
f tb	2,583	15	0.6
f fu	2,953	99	3.4
f bb	1,724	184	10.7

verted as compared with that of *melanogaster* (*see* Sturtevant and Plunkett, 1926). This was the first inverted section that was demonstrated, and it was tempting to suppose that the inversion was in some way connected with the specific distinctness of *melanogaster* and *simulans*. This supposition has become very much less probable with the discovery (Sturtevant 1926 and unpublished data) that similar inversions of sections of chromosomes-II and III are not uncommon within the species *melanogaster*, and that they do not there result in visible somatic differences or cross-sterility.

TABLE 5—*Summary of all linkage data on chromosome-II*

Loci	Total flies	Crossovers	Per cent
T b	699	271	38.9
T py	632	319	50.5
T sd	632	317	50.2
T pm	699	347	49.6
dh b	900	340	37.8
dh py	157	80	51.0
dh sd	900	429	47.6
dh pm	743	389	52.4
b a-w	591	51	8.6
b S-a	344	66	19.2
b sw	1,099	212	19.3
b vg^n	127	43	33.9
b py	4,113	1,290	31.4
b sd	5,131	1,760	34.3
b pm	7,782	3,580	46.0
a-w py	321	92	28.7
a-w pm	652	286	43.9
S-a sd	242	59	24.4
S-a pm	242	119	49.2
sw sd	822	206	25.1
sw pm	1,713	767	44.7
vg^n pm	127	33	26.0
py sd	2,390	138	5.8
py pm	3,956	1,225	31.1
sd pm	11,160	2,580	23.1

TESTS FOR CROSSOVER MODIFIERS IN WILD STOCKS

Recent observations (Sturtevant 1926 and unpublished data) have shown that variations in the sequence of loci ("inverted sections") are not infrequent in wild stocks of *melanogaster,* though the sequences shown by the standard maps are by far the most commonly found. It is therefore of some interest to discover how regularly the *simulans* sequence agrees with the maps here given. In particular, how constant are the resemblances to *melanogaster,* and more especially the differences from it?

TABLE 6—*Summary of all linkage data on chromosome-III*

Loci	Total flies	Crossovers	Per cent
cu st	1,387	479	34.6
cu Δ	1,715	742	43.3
cu p	1,912	922	48.2
cu rg	642	331	51.6
se rh-b	409	76	18.6
se st	937	257	27.4
se Δ	773	346	44.7
rh-b st	1,543	249	16.1
rh-b H	359	124	34.6
rh-b p	1,134	509	44.9
d-l st	646	90	13.9
d-l Δ	1,225	414	33.8
d-l p	2,979	1,349	45.2
st H	3,657	677	18.6
st Δ	9,478	2,253	23.7
st ap	1,150	354	30.8
st bp	801	253	31.6
st p	11,654	5,050	43.4
st rg	1,604	732	45.7
st M	1,277	590	46.2
st ca	230	104	45.2
H Δ	2,833	74	2.6
H ap	652	143	21.8
H p	3,591	1,271	35.4
Δ ap	791	81	10.2
Δ bp	1,861	258	13.9
Δ p	10,529	3,828	36.4
Δ rg	708	275	38.8
Δ M	1,420	651	45.8
Δ ca	230	100	44.5
ap p	293	73	24.9
bp p	1,094	305	27.9
p rg	2,073	56	2.7
p M	1,552	440	28.3
M ca	75	0	0

General experience has been that crossover values are relatively constant in *simulans.* There has been no case that in any way suggested an alteration of the sequence of any of those loci also known in *melanogaster,* except perhaps the CII described below. A reducer of crossing over in the left end of the X is described in this paper; and a small series of data dealing with the right end of chromosome-III has been omitted because it gave somewhat reduced crossover values. These and the one case described below are the only inherited crossover variations observed. However, it seemed desirable to examine the point specifically in a few wild stocks.

A few small series of tests have been carried out for this purpose. The dominant reducer of crossing over in the left end of the X is described elsewhere in this paper, and it is there shown that the following wild stocks all gave similar crossover values when crossed to the "reduced" strain: Woods Hole, Mass.; Morristown, N. J.; Rochester, Minn.; St. Augustine, Fla.; and Kushla, Ala.

Only one specific test was made for chromosome-II. Multiple recessive stocks were crossed to a wild stock collected at New Orleans, La., in 1926. The F_1 females gave approximately normal values for the intervals from black to plum; but to the left of black crossing over was absent or at least markedly reduced. Owing to the fact that truncate and dash, the two mutants whose loci lie to the left of black, are both unsatisfactory characters in that they overlap wild-type in appearance, this "CIIL" has not been studied in detail. It may represent an inverted section; if so it is the only one yet met with in *simulans.*

The third chromosome has been more extensively tested. A scarlet hairless peach stock was mated to wild stocks from Rochester, Minn. (1919), and from Kushla, Ala. (1921). A sepia scarlet hairless stock was mated to wild stocks from Woods Hole, Mass. (1922), Morristown, N. J. (1923), Rochester, Minn. (1919), New Orleans, La. (1926), Kushla, Ala. (1921, 1924), and St. Augustine, Fla. (1923). Tests of the F_1 females from all these matings gave substantially normal crossover values. It follows that the sequence shown on the map of chromosome-III is normal for the species, and does not represent a mere occasional difference from the *melanogaster* sequence.

AN INHERITED LINKAGE VARIATION IN THE X-CHROMOSOME

One series of crosses has been omitted from the general summary of linkage data on the X-chromosome, because it obviously shows values belonging to a different system. A stock of yellow prune dusky forked was made up, and was crossed to various wild-stocks (from St. Augustine, Florida; Kushla, Alabama; Morristown, New Jersey; Rochester, Minnesota; Woods Hole, Massachusetts). All the F_1 females from these matings gave crossover values for yellow prune that were significantly lower than the usual value of 2.9, shown in table 4. The total counts are shown in table 7. (*See* introductory statement to Appendix for method of tabulating data.)

TABLE 7—*Reduced crossing over, y pn dy f*

Type of cross	0	1	2	3	1, 2	1, 3	2, 3	Total
y pn dy f × +.....	1,379 1,172	16 20	694 584	510 454	0 2	8 7	175 114	5,135
y pn × dy f.......	326 316	5 5	184 195	115 88	4 1	1 2	52 30	1,324

The data in the second row of the table were obtained by mating together yellow prune and dusky forked crossovers from the experiments recorded in the first row, and testing the wild-type daughters that were produced. Some of the data in the first row were obtained by testing wild-type females produced by earlier cultures of the same series. All females of these two kinds that were tested gave the reduced crossover values. No further tests were made; but the data are sufficient to establish the existence of a dominant crossover reducer in the y pn dy f stock, and to suggest that this reducer was itself in the X-chromosome. The data of table 7 give the following crossover values:

	Table 7	Standard (Table 4)
y pn	1.1	2.9
pn dy	31.5	39.3
dy f	24.1	21.7

The corresponding values for the *melanogaster* X are, approximately, 1.0, 37, and 20. It will be seen that the reduced y pn value of *simulans* is much like the *melanogaster* value, but that for the other two intervals the standard values are closer to the *melanogaster* value.

THE EFFECTS OF AGE ON CROSSING OVER

A few experiments have been carried out to test the effects of age of female on crossing over in her eggs. The results are shown in tables 8 and 9. In these experiments, a newly emerged heterozygous female was mated to multiple recessive males and placed in a vial. At the end of three days these flies were transferred to a new vial, and thereafter were transferred again every second day. The offspring produced in the first vials are recorded in the rows labelled "a" in the tables, those from the second vials are entered in the rows labelled "b," etc. Each value in the table is the total obtained from several sister females tested at the same time and recorded separately: the raw data have been studied to see if significant differences between sister females occurred, but no such differences have been found. The temperature in all cases was about 25°C.

One may conclude that no appreciable age change occurs in the region tested in chromosome-II. In all three experiments with chromosome-I, a slight rise in crossing over during the first 7 or 8 days is indicated, perhaps followed by a decrease later. The data are not extensive enough to make this conclusion certain; the fact that all three values follow roughly the same course in all three experiments is strongly indicative of a significant relation, but this indication is weakened by the fact that experiments II and III belong to a system of crossing over different from the "standard" type represented by experiment I.

It should be noted that none of the regions here studied have been found to be sensitive to age change in *melanogaster*.

TESTS FOR CROSSING OVER IN THE MALE

The experiments previously recorded (Sturtevant 1921*b*) indicated that there is no crossing over in the autosomes of the *simulans* male. Such a relation is certainly the usual one, and has constantly been taken for granted in planning experiments and keeping stocks. All the results from such matings have been uniformly consistent with the absence of crossing over in males. A few direct tests of the relation have been made, by back-crosses to multiple recessives. These results appear in table 10.[1]

TABLE 8—*Effect of age of female on crossing over in the X-chromosome*

	Experiment I				Experiment II				Experiment III			
	N	1	2	3	N	1	2	3	N	1	2	3
a	267	9.7	20	21	118	0	25	21	520	1.2	28	22
b	387	8.8	22	23	281	0.7	29	23	540	0.9	35	23
c	404	11.6	30	24	417	1.4	32	23	528	1.7	38	24
d	259	11.6	29	24	426	1.6	39	22	441	1.1	37	28
e	352	9.7	28	26	408	1.0	37	21	487	1.4	32	25
f	129	7.8	30	22	330	0.6	37	23	309	0.3	28	27
g	182	12.1	25	21	....	....	....	....	370	1.4	32	24
h	150	8.7	29	29	....	....	....	....	152	2.0	32	20

Experiment I—y × rb dy f.
Experiment II—y pn dy f × +; reduced crossing over system.
Experiment III—same type as II.

One crossover appears in this table. It came from a mating of st p ♀ × Δ/ st p ♂, that gave 116 Δ, 67 st p, and one p ♀. This peach female was mated to scarlet peach brothers, and gave 59 p and 52 st p offspring. Tests of the descendants of these 59 p flies showed that the p chromosome of the original exceptional female carried p, the wild-type allelomorph of st, and a lethal. These three were all separable, but their exact loci were not determined. Neither was it determined whether the lethal was already present in the Δ/ st p male of the original culture. It remains uncertain

TABLE 9—*Effect of age of female on crossing over in chromosome-II*

(♀ sd pm /+ × ♂ sd pm.)		
	N	Per cent crossing over
a	969	28.5
b	834	25.5
c	544	27.9
d	536	27.6
e	374	27.0
f	367	31.1
g	341	29.1
h	361	24.2
i	220	29.1

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[1] These data include all those previously published, except that a small series (24 flies) from scarlet roughish has been omitted here. See introductory statement to Appendix for Method of Tabulating Data.

whether the exceptional individual of table 10 represents crossing over in a male (between st and △), or reversion of scarlet.[1]

In any case, crossing over in the autosomes of the *simulans* male is exceedingly rare, if not entirely absent.

INDEPENDENT INHERITANCE OF THE FOUR GROUPS OF GENES

The independence of the four linkage groups has not been studied systematically, either in *melanogaster* or in *simulans*. That complete independence is the rule can scarcely be doubted, but few specific data on the point are available. In the case of the female, the occurrence of crossing over within each of the first three groups makes a complete demonstration very laborious; and in fact such a detailed demonstration has not been made in either species. In the male, however, it suffices to test one pair of genes in each group, since there is no crossing over within a group. Such a test, involving all four linkage groups at once, was described for *melanogaster* by Muller (1914).

TABLE 10—*Backcross tests of heterozygous males*

Chromosome	Loci	Type of cross	Non crossovers		Crossovers		Total
II............	b pm	b pm × +	151	112	0	0	263
II............	sd pm	sd pm × +	313	298	0	0	611
III............	st p	st p × +	527	381	1	0	909
		st × p	343	335	0	0	678

One such experiment has been carried out with *simulans*, using sex to control chromosome-I, polychæte for II, scarlet for III, and minute-4 for IV. Males of two different types were mated to polychæte scarlet females. In the first series the males had received a polychæte II and normal X, III, and IV from their mothers; Y, a normal II, a scarlet III, and a minute-4 IV from their fathers. In the second series they had X, polychæte, scarlet, and a normal IV from their mothers; Y, minute-4, and normal II and III from their fathers. The results are shown in table 11.

Analysis of these results shows that, among the 877 flies, the following new combinations occurred:

I and II, 443 (50.6 per cent)
I and III, 429 (48.7 per cent)
I and IV, 433 (49.4 per cent)
II and III, 478 (54.6 per cent)
II and IV, 434 (49.5 per cent)
III and IV, 430 (48.8 per cent)

These results are all clearly to be interpreted as random deviations from 50 per cent, though that for II and III is near the border of statistical significance. In this instance, however, the two experiments of table 11 do not agree in showing a similar deviation, and the observed ratio is such that more new combinations arose than old ones. There can be no doubt that

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[2] It may be noted that the one exceptional individual recorded by Sturtevant and Plunkett (1926) from *melanogaster* △H/+ by *simulans* △/H (see discussion of delta in this paper) may be interpreted as resulting from crossing over in the *simulans* male between △ and H.

the result is due to chance, and that all four linkage groups are fully independent in the male of *simulans.*

RELATIVE FREQUENCY OF MUTATION IN *MELANOGASTER* AND *SIMULANS*

The total frequency of mutation in a species of *Drosophila* cannot be determined, since the detection of mutations involves so large a personal equation. However, most investigators who have studied several species have been inclined to believe that mutations were more frequent in some than in others. This is my own impression (*e.g.,* that *busckii* and *funebris* have a relatively low frequency, *immigrans* a relatively high one). My opinion is, however, that *melanogaster* and *simulans* have much the same mutation frequency.

In the case of *melanogaster* and *simulans* it is possible to investigate a related question: are the relatively highly mutable genes of one species also mutable in the other one? Morgan, Bridges, and Sturtevant (1925) have given a table showing the loci that are known to have mutated more than once in *melanogaster.* Comparison of this table with the data in the present paper brings out several points. Twenty-seven of the loci that have mutated in *simulans* have also mutated in *melanogaster.* This is, roughly, 8 per cent of the known *melanogaster* loci and 50 per cent of the known in *simulans.* Of these 27, 18 have mutated more than once in *melanogaster,*[1] and five have recurred in *simulans.*

Table 11—*Tests for independence of four linkage groups in the male*

Male	M ♀	py st ♂	+ ♀	py st M ♂	st M ♀	py ♂	st ♀	py M ♂	py M ♀	st ♂	py ♀	st M ♂	py st M ♀	+ ♂	py st ♀	M ♂	Total
$\frac{X}{Y}\frac{py}{+}\frac{+}{st}\frac{+}{M4}$	43	42	49	38	33	30	37	31	26	35	40	33	36	59	35	49	616
$\frac{X}{Y}\frac{py}{+}\frac{st}{+}\frac{+}{M4}$	17	17	18	14	13	26	19	16	8	16	12	17	9	23	19	17	261

Of the five recurrent types of *simulans* (yellow, white, ruby, singed, bobbed), each has mutated at least five times in *melanogaster.* Of the 22 types that have occurred five or more times in *melanogaster,* 13 have occurred in *simulans*—60 per cent, as against the 8 per cent for all *melanogaster* mutant loci. These figures leave no doubt that high mutability of a locus in one species is correlated with high mutability of that locus in the other species.

Several loci in *melanogaster* show a mutability that is extraordinarily high as compared to the general frequency curve for mutability in that species. Notch and white are especially noteworthy in this respect: white has occurred twice in *simulans,* notch has not been found. Yellow, cut, vermilion, and rudimentary form the group next highest in frequency in *melanogaster;* cut and vermilion have not been found in *simulans,* rudimentary has occurred once, yellow twice. It is still doubtful if the absence of notch, cut and vermilion is significant.

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[1] More recent data indicate that prune, straw, spineless, and hairless have recurred in *melanogaster,* so that at least 22 of the 27 are recurrent types in *melanogaster.*

NON-DISJUNCTION—CHROMOSOME-I

Non-disjunction of the sex chromosomes occurs in *simulans* as in *melanogaster* (Sturtevant 1921*a*). As stated elsewhere in this paper, primary non-disjunction is relatively frequent in females homozygous for claret. Because of this high frequency it has been possible to show that males arising as primary exceptions are sterile. They are accordingly XO in constitution, as expected; and XO males are likewise shown by this evidence to be sterile in *simulans* as they are in *melanogaster* (Bridges 1916, Safir 1920). Females arising as primary exceptions are sometimes "equationals"—*i.e.*, homozygous for certain loci for which their mothers were heterozygous. A study of these equationals shows the same relations as in *melanogaster:* the frequency of equational exceptions is greatest for loci nearest the left (yellow) end of the X-chromosome, lowest for loci nearest the right (bobbed) end of the X. This relation clearly means that the X-chromosome is attached to the spindle-fiber at its right end (*see* analysis for *melanogaster* by Anderson 1925 and Bridges and Anderson 1925).[1]

Exceptional females of *simulans* are XXY in constitution, as expected, since they give further (secondary) exceptions. Among these secondary exceptions, males and females are about equally frequent, and the males are fertile. It has also been found that some of the regular daughters of exceptional females produce exceptions. These relations agree so closely with those found by Bridges (1916) in *melanogaster* that there can be no doubt as to the identity of the mechanism concerned.

The frequency of secondary exceptions is rather less in *simulans* than in *melanogaster*—2.9 per cent and 0.9 per cent in the two series studied.

Two additional classes of zygotes should be produced by XXY females—YY and XXX. The first may safely be assumed to be inviable. The second (XXX) sometimes survives in *melanogaster* as a sterile individual of a definite somatic type known as superfemale (Bridges 1925). Such individuals have not been certainly identified in *simulans;* but no cultures have been available in which they should have occurred in large numbers, so the most that can be concluded is that they are not fully viable.

NON-DISJUNCTION—CHROMOSOME-IV

About 40 per cent of the offspring of claret females belong to a definite small-bristled type, and another 4 per cent show this same character in part but not all of their bodies. Analogy with *melanogaster* indicated that this special type of fly was due to loss of one member of the pair of fourth chromosomes; and direct cytological examination has verified this conclusion.

The haplo-IV flies have small bristles, the distal section of the fourth vein is imperfect, they develop slowly, and both sexes are usually sterile.

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[1] The detailed account of the experiments on non-disjunction in claret females will be published in connection with the other results from claret.

They lack the well-marked "trident" pattern on the mesonotum that is characteristic of haplo-IV *melanogaster* (Bridges 1921); but this is to be correlated with the fact that such a trident is regularly completely absent in wild-type *simulans*.

Many attempts have been made to breed haplo-IV *simulans*. A total of three females and two males have produced offspring; in every case the output, though very small, included a few haplo-IV specimens. It has not been possible to carry out any experiments with the type, or to keep a stock of it. It can, however, always be produced in quantity by breeding from claret females.

The triplo-IV type has not been recognized in *simulans*, though it is probably present in small numbers among the offspring of claret females.

MOSAICS

Six mosaics, probably due to elimination of X-chromosomes, have been seen among *melanogaster-simulans* hybrids. In the four cases where the point could be determined, it was the *melanogaster* X that was eliminated; one may surmise that hybrid tissue without a *simulans* X is inviable when it forms part of a mosaic, as it is when it forms a whole individual.

More than thirty mosaics (in addition to those described (Sturtevant 1921) and to those coming from claret mothers) have been found in miscellaneous *simulans* cultures. The majority of these can be easily interpreted as due to elimination of X-chromosomes; it does not seem necessary to describe them or to record their dates and pedigrees, as they add nothing significant to what is known about *Drosophila* mosaics. There are, however, five exceptional individuals that may be discussed here.

1. A female of the constitution rb/vs f was mated to rb males. Among the offspring there was found a mosaic, male throughout. The head, front legs, right side of thorax and abdomen, external genitalia, left dorsocentrals, scutellars, postalars, supra-alars, and upper humeral, and the left sides of the first three abdominal segments, were ruby, not-vesiculated, not-forked; the remainder of the external parts were vesiculated forked. Both types of tissue could be identified as male. Internally, the specimen was a normal male; it was fertile, and bred as a ruby (not-vesiculated, not-forked) male.

There was a Y present in the testes, since XO males are sterile in *simulans*. Accordingly, it is probable that the rb X of the mosaic came from its mother, as the vs f X certainly did. A double-nucleus egg seems the simplest interpretation.

2. A female of the constitution p/st p was mated to a male st/p. One of the resulting daughters had one scarlet eye and one scarlet peach eye. She was not tested genetically. Since scarlet peach was not expected to occur in this culture, and was not present in any of the sibs, elimination of that part of chromosome-III carrying not-p seems the simplest interpretation (*see* Stern 1927*b*).

3. A roughish female was mated to a scarlet delta peach male. One of the offspring was a gynandromorph that was delta on the male (right) side, and not-delta on the female side. The abdomen was wholly male; the testes were small, and the specimen was sterile. Since this fly was a mosaic both for chromosome-I and for chromosome-III, it seems probable that two sperms took part in its formation. The sterility remains unexplained.

4. A female of the constitution Δ/st p M was mated to peach males. One son was wild-type, except that each eye had a peach region covering rather more than half its area. The mosaic was fertile, and bred as a st/p male. As in the specimen recorded above as number 2, the most probable interpretation seems to be the elimination of the not-p section of chromosome-III. The double-nucleus interpretation suggested for number 1 is also possible here. Another possibility is the occurrence of a somatic mutation to a sex-linked recessive eye-color resembling peach.

5. A stated in describing the origin of singed, the culture that gave the original singed male also gave a mosaic. A single female, that had black in one second chromosome and polychæte in the other, was mated to black polychæte spread males. One mosaic was produced that was polychæte in the female parts and wild-type in the male parts. The entire head and the left side of the thorax and abdomen were polychæte and female; the right side of thorax and abdomen and the external genitalia were wild-type male (sex-comb on right side only). This individual is easily accounted for on the assumption of two separately fertilized egg-nuclei. No other simple interpretation has occurred to me.

GYNANDROMORPHS FROM CLARET

As stated in describing the claret mutant type, about three per cent of the offspring of claret females are gynandromorphs, in which a maternal X-chromosome has been eliminated at an early cleavage division. A detailed study has been made of the number and distribution of the resulting male parts in a series of 96 such gynandromorphs from claret mothers.

This study indicates that elimination occurs at the first cleavage division in roughly 40 per cent of these gynandromorphs, at the second division in roughly 20 per cent, at the third or later in roughly 25 per cent, and at more than one division (probably usually the first and a later one) in roughly 15 per cent. These numbers are, however, only first approximations.

The data have also been used in a study of the cell-lineage of the surface of the adult fly. In general, the conclusions are that there is no definite cleavage pattern, and that the fate of a cleavage nucleus is determined by the position in which it comes to lie rather than by any property of the nucleus itself.

The cells of the imaginal discs proliferate freely and without any predetermined pattern; there is no subsequent mingling of cells from different origins, so that male and female portions occur (in the gynandromorphs) in definite sharply bounded areas. Some of the imaginal discs give rise to specific regions of the adult and to no others, while other discs are more variable. The wing-disc (dorsal mesothoracic) shows both relations: it never contributes to the humeral area (that comes from the dorsal prothoracic disc), and the boundary between regions descended from right and left wing-discs always lies in the median dorsal line of the fly (between the two middle rows of acrostichal hairs). But the sternopleural region is sometimes supplied by the wing-disc and sometimes by the disc of the second leg (ventral mesothoracic). In the latter case, as in the embryo, the result is evidently determined not by the past history or internal properties of the cells concerned, but by the position they come to occupy in the developing pupa.

LITERATURE CITED

ANDERSON, E. G. 1925. Crossing over in a case of attached X-chromosomes in *Drosophila melanogaster*. Genetics 10:403–417.

BONNIER, G. 1924. Contributions to the knowledge of intra- and inter-specific relationships in *Drosophila*. Acta Zool. 5:1–122.

BRIDGES, C. B. 1916. Non-disjunction as proof of the chromosome theory of heredity. Genetics 1:1–52, 107–163.

———. 1921. Genetical and cytological proof of non-disjunction of the fourth chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. 7:186–192.

———. 1925. Sex in relation to chromosomes and genes. Amer. Nat. 59:127–137.

———, and E. G. ANDERSON. 1925. Crossing over in the X-chromosomes of triploid females of *Drosophila melanogaster*. Genetics 10:418–441.

EPHRUSSI, B. 1925. Sur le chondriome ovarien des *Drosophila melanogaster* et *simulans*. C. R. Soc. Biol. 92:778–780.

EVANG, K. 1925. The sex-linked mutants vesiculated and semi-lethal in *Drosophila melanogaster*. Zeits. ind. Abst. Vererb. 39:165–183.

JOHNSON, C. W. 1913. The distribution of some species of *Drosophila*. Psyche 20: 202–204.

LANCEFIELD, D. E. 1922. Linkage relations of the sex-linked characters in *Drosophila obscura*. Genetics 7:335–384.

LYNCH, C. J. 1919. An analysis of certain cases of intra-specific sterility. Genetics 4:501–533.

METZ, C. W., and C. B. BRIDGES. 1917. Incompatibility of mutant races in *Drosophila*. Proc. Nat. Acad. Sci. 3:673–678.

———, M. S. MOSES, and E. D. MASON. 1923. Genetic studies on *Drosophila virilis* with considerations on the genetics of other species of *Drosophila*. Carnegie Inst. Washington, Publ. 328, 94 pp.

MOHR, O. L. 1923. A genetic and cytological analysis of a section deficiency involving four units of the X-chromosome in *Drosophila melanogaster*. Zeits. ind. Abst. Vererb. 32:108–232.

MORGAN, T. H., C. B. BRIDGES, and A. H. STURTEVANT. 1925. The genetics of Drosophila. Bibliogr. Genetica 2:1–262.

MULLER, H. J. 1914. A gene for the fourth chromosome of *Drosophila*. Journ. Exper. Zool. 17:325.

PLUNKETT, C. R. 1926. The interaction of genetic and environmental factors in development. Journ. Exper. Zool. 46:181–244.

QUACKENBUSH, L. S. 1910. Unisexual broods of *Drosophila*. Science, n.s., 32:183–185.

SAFIR, S. R. 1920. Genetic and cytological examination of the phenomena of primary non-disjunction in *Drosophila melanogaster*. Genetics 5:459–487.

STERN, C. 1927*a*. Ein genetischer und zytologischer. Beweis für Vererbung im Y-Chromosom von *Drosophila melanogaster*. Zeits. ind. Abst. Vererb. 44:187–231.

———. 1927*b*. Über Chromosomenelimination bei der Taufliege. Die Naturwiss. 15: 740–746.

STURTEVANT, A. H. 1915. Experiments on sex recognition and the problem of sexual selection in *Drosophila*. Journ. Animal Behav. 5:351–366.

———. 1919. A new species closely resembling *Drosophila melanogaster*. Psyche 26: 153–155.

———. 1920*a*. Intersexes in *Drosophila simulans*. Science, n.s., 51:325–327.

———. 1920*b*. Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *D. melanogaster*. Genetics 5:488–500.

———. 1921*a*. Genetic studies on *Drosophila simulans*. II. Sex-linked group of genes. Genetics 6:43–64.

———. 1921*b*. Genetic studies on *Drosophila simulans*. III. Autosomal genes. General discussion. Genetics 6:179–207.

———. 1921*c*. The North American species of *Drosophila*. Carnegie Inst. Washington, Publ. 301.

———. 1921*d*. A case of rearrangement of genes in *Drosophila*. Proc. Nat. Acad. Sci. 7:235–237.

———. 1926. A crossover reducer in *Drosophila melanogaster* due to inversion of a section of the third chromosome. Biol. Zentralbl. 46:697–702.

———, and C. R. PLUNKETT. 1926. Sequence of corresponding third-chromosome genes in *Drosophila melanogaster* and *D. simulans*. Biol. Bull. 50:56–60.

APPENDIX

SUMMARY OF NEW LINKAGE DATA

Tables 12 to 22 give the linkage data on *simulans* accumulated since the first general accounts of the species (Sturtevant 1921*a* and 1921*b*) were published. These new data and those of the two papers mentioned are combined and summarized in tables 4 to 6, in the text of this paper.

The data are here presented in the same form as in the earlier accounts. The first column of each table shows not only the loci concerned, but their sequence. In the third and following columns, classes are entered under headings that indicate the intervals within which they represent crossing over. There are, of course, two contrary classes representing each type of crossing over; of these two, that one which carries the dominant allelomorph in the left-most locus concerned is always entered first in the table.

Table 12—*Two-point experiments with the X-chromosome*

Loci	Type of cross	Non-crossovers		Crossovers		Total
y E	y × E	25	147	7	1	180
y w	y × w	529	513	24	22	1,088
y fa	y × fa	235	224	10	3	472
y sn	y × sn	118	107	34	22	281
E rb	E × rb	102	250	12	19	383
rb dy	rb dy × +	125	126	31	34	316
	rb × dy	390	346	134	145	1,015
rb tb-b	rb × tb-b	311	301	119	95	826
cv fu	cv × fu	554	788	579	98	2,019

TABLE 13—*Three-point experiments with the X-chromosome*

Loci	Type of cross	Non-crossovers		Single crossovers				Double crossovers		Total
				Region 1		Region 2				
y pn dy.......	y pn × dy	257	202	6	10	173	129	1	3	781
y pn E........	y pn × E	196	488	13	10	1	0	0	0	708
y pn tb-b......	y pn × tb-b	70	66	2	1	55	36	2	0	232
y w fa	y w × fa	469	481	30	23	12	12	0	0	1,027
y w sn........	y sn × w	131	180	8	5	37	48	0	0	409
y w fu........	y fu × w	124	77	9	12	82	105	1	2	412
y fa rb........	y × fa rb	241	242	9	9	3	9	0	0	513
y rb cv.......	y rb cv × +	605	448	46	50	32	27	1	1	1,210
	y × rb cv	388	520	66	33	20	15	0	0	1,042
y rb sn	y sn × rb	258	276	34	30	55	52	0	1	706
y rb dy.......	y dy × rb	60	85	20	8	33	29	2	4	241
y rb tb-b......	y tb-b × rb	52	44	6	2	11	19	2	0	136
y rb fu........	y fu × rb	155	127	30	25	96	111	6	5	555
y cv sn........	y × cv sn	148	166	47	39	16	18	1	0	435
y vs g.........	y g × vs	76	...	...	30	10	...	...	2	118
y vs f.........	y f × vs	112	...	...	30	64	...	...	10	216
y g tb-b	y g × tb-b	230	270	142	113	2	1	0	0	758
y g f..........	y g × f	121	126	77	87	23	16	5	8	463
y tb-b f.......	y f × tb-b	88	...	...	42	...	15	4	...	149
y r f..........	y f × r	59	...	...	49	2	...	...	0	110
w vs sn........	w sn × vs	68	...	...	6	5	...	...	0	79
w sn dy.......	w × sn dy	100	69	23	25	18	15	4	2	256
fa rb dy.......	fa dy × rb	115	118	4	6	53	43	0	0	339
rb cv dy......	rb dy × cv	366	391	12	12	49	76	0	1	907
	rb × cv dy	100	119	11	0	29	39	1	0	299
rb vs f........	rb × vs f	134	...	...	16	56	...	...	4	210
rb sn dy......	rb dy × sn	23	28	6	4	3	9	0	0	73
	rb × sn dy	143	142	35	24	34	21	5	1	405
rb dy bb......	rb dy × bb	65	...	...	19	...	16	6	...	106
cv sn dy.......	cv dy × sn	293	278	24	26	64	51	0	0	736
vs g f.........	vs × g f	...	133	...	56	...	18	...	8	215
sn dy g.......	sn dy g × +	232	179	24	45	23	24	1	0	528
	sn dy × g	184	225	49	40	24	13	3	2	540
dy g f.........	dy × g f	764	897	68	50	176	173	0	2	2,130
dy f bb........	dy f × bb	122	...	...	48	...	20	2	...	192
g r f..........	g f × r	84	...	...	7	2	...	...	0	93
g f fu.........	g f × fu	414	485	74	73	22	16	1	0	1,085
g f bb.........	g f × bb	291	...	...	39	...	44	1	0	375

TABLE 14—*Four-point experiments with the X-chromosome*

Loci	Type of cross	Non-crossovers		Single crossovers						Double crossovers						Triple crossovers		Total
				Reg. 1		Reg. 2		Reg. 3		1, 2		1, 3		2, 3				
y pn dy f..	y dy f × pn	78	44	3	3	48	37	35	29	0	1	0	2	7	14	0	0	301
y w fa sn..	y w sn × fa	73	85	8	5	3	2	20	31	0	0	0	1	0	0	0	0	228
y w rb cv..	y rb cv × w	...	283	17	...	...	23	...	22	0	..	0	..	..	0	0	..	345
y w sn dy..	y × w sn dy	43	69	1	2	15	18	15	13	0	0	1	1	1	3	0	0	182
y w dy f..	y w dy f × +	100	73	4	5	48	50	19	29	0	0	0	0	8	5	0	0	341
	y w f × dy	30	46	0	4	25	25	7	8	1	0	0	1	2	7	0	0	156
	y w × dy f	353	325	22	18	229	215	120	111	3	3	6	7	45	71	1	1	1,530
y fa rb sn-2.	y × fa rb sn-2	241	294	12	16	7	3	18	16	0	0	0	0	1	0	0	0	608
y fa rb dy.	y fa dy × rb	115	107	7	5	1	4	44	29	0	0	1	2	0	1	0	0	316
y rb cv sn.	y rb cv × sn	583	471	48	54	35	33	58	32	1	0	0	2	0	0	0	0	1,317
y rb dy f.	y rb dy f × +	290	249	40	52	126	144	115	96	4	3	19	27	33	27	0	0	1,225
	y dy f × rb	219	174	43	49	97	94	60	88	6	4	7	10	18	25	3	0	897
	y f × rb dy	158	171	29	25	63	78	48	58	12	4	7	7	9	9	2	0	680
y cv g f...	y cv × g f	129	92	25	27	67	44	22	24	8	4	2	3	7	6	0	0	460
y dy g f..	y dy g f × +	230	253	118	145	19	26	44	51	4	1	19	12	1	0	0	0	923
	y dy g × f	125	111	58	68	0	9	33	9	0	2	9	8	1	1	0	0	434
	y dy × g f	240	230	148	143	13	17	55	53	9	9	27	16	0	0	0	0	960
	y g × dy f	216	206	113	113	15	21	43	40	6	3	19	13	2	0	0	0	810
	y f × dy g	43	68	37	31	3	7	9	10	2	5	8	8	0	0	0	0	231
	y × dy g f	139	176	108	76	11	20	35	30	2	0	10	11	0	0	0	0	618
y dy f fu..	y fu × dy f	190	191	119	116	69	56	6	4	32	32	5	3	1	0	0	0	824
y g r f....	y g r f × +	...	53	43	...	12	...	...	2	..	3	0	..	0	..	..	0	113
	y g × r f	85	...	...	39	...	8	0	...	2	..	..	1	..	0	0	..	135
	y r × g f	...	44	16	...	5	0	...	1	..	0	1	..	0	..	..	0	67
y g f fu....	y × g f fu	51	101	68	36	36	16	6	5	7	11	1	2	0	0	0	0	340
fa rb dy f..	fa rb × dy f	169	163	3	6	68	58	58	43	0	1	1	7	6	11	0	0	594
cv dy g f..	cv dy × g f	345	273	120	88	48	16	86	49	2	1	12	5	0	0	0	0	1,045
dy g f bb..	dy bb × g f	...	119	17	...	20	...	13	...	..	0	..	0	..	2	0	..	171

TABLE 15—*Five-point experiments with the X-chromosome*

Loci	Type of cross	Non-cross-overs		Single crossovers								Double crossovers												Triple crossovers								Total
				Region 1		Region 2		Region 3		Region 4		1,2		1,3		1,4		2,3		2,4		3,4		1,2,3		1,2,4		1,3,4		2,3,4		
y pn fa dy f.......	y pn × fa dy f	55	81	1	3	7	2	43	41	23	24	0	0	1	0	0	0	1	1	2	0	13	12	0	0	0	0	0	0	0	0	310
y w fa dy f.......	y w fa dy f × +	143	107	6	3	1	0	88	65	37	31	0	0	0	0	1	1	1	0	2	1	9	10	0	0	0	0	0	0	0	0	506
	y w fa × dy f	218	171	9	11	2	5	150	99	75	46	0	0	3	5	2	0	2	1	3	2	24	34	0	0	0	0	0	1	1	0	864
	y × w fa dy f	123	167	10	8	8	0	64	84	50	62	0	0	0	0	3	1	0	1	2	2	9	11	0	0	0	0	0	0	0	0	605
y w dy f fu........	y w dy f × fu	106	97	5	11	60	51	26	23	3	4	1	3	0	1	0	1	10	12	6	3	1	0	0	0	0	1	0	0	0	0	425
y w dy f bb.......	y w × dy f bb	66	56	3	1	52	38	17	22	11	10	1	1	1	2	0	0	12	9	7	6	3	2	0	0	0	0	0	0	0	0	320
y fa rb sn-2 dy....	y dy × fa rb sn-2	257	260	9	8	14	9	22	23	31	44	0	0	0	0	1	0	0	0	1	1	2	1	0	0	0	0	0	1	1	0	685
y fa rb dy f.......	y × fa rb dy f	72	145	8	5	1	2	29	41	26	44	0	0	0	2	2	1	0	0	2	1	5	9	0	0	0	0	1	0	0	0	396
y rb cv sn dy.....	y rb cv × sn dy	107	119	14	20	8	7	12	10	21	16	0	0	2	1	2	2	0	0	0	0	1	2	0	0	0	0	0	0	0	0	344
	y cv sn × rb dy	37	37	1	3	4	3	3	3	10	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	105
y rb cv dy f.......	y rb cv × dy f	94	90	12	6	6	3	35	38	35	24	0	0	1	1	5	2	0	0	1	0	8	3	0	0	0	0	0	0	1	0	365
y rb dy f bb......	y dy f × rb bb	135	..	..	15	..	62	..	55	..	16	2	..	6	..	1	..	14	..	10	..	0	..	..	0	..	1	..	2	..	0	319
	y × rb dy f bb	58	...	..	2	..	18	..	21	..	13	0	..	4	..	2	..	1	..	3	..	0	..	..	1	..	0	..	0	..	0	123
y dy g f fu........	y dy × g f fu	58	83	40	34	12	5	9	13	5	0	1	4	7	5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	279
y dy g f bb........	y dy bb × g f	...	89	71	..	17	..	19	..	11	..	..	0	..	13	..	3	..	0	..	1	..	0	0	..	0	..	0	..	0	..	224

TABLE 16—*Two-point experiments with chromosome-II*

Loci	Type of cross	Non-crossovers		Crossovers		Total
b a-w.........	b × a-w,......	136	109	15	10	270
b S-a.........	b S-a × +	.....	86	16	...	102
a-w pm.......	a-w pm × +	96	91	68	76	331
sd pm.........	sd pm × +	1,092	1,022	281	267	2,662

TABLE 17—*Three-point experiments with chromosome-II*

Loci	Type of cross	Non-crossovers		Single crossovers				Double cross-overs		Total
				Region 1		Region 2				
T b pm......	T × b pm	19	...	15	...	22	...	11	..	67
b sw pm......	b pm × sw	191	196	41	48	161	194	34	26	891
b vg^{n} pm	b vg^{n} × pm	...	57	37	...	...	27	6	..	127
b py pm.....	b py pm × +	222	204	107	100	114	98	33	35	913
	b × py pm	119	113	45	45	59	67	22	19	489
b sd pm.....	b sd pm × +	278	229	138	129	121	95	29	38	1,057
	b × sd pm	108	117	77	62	46	47	22	12	491
sw sd pm....	sw sd pm × +	57	52	22	17	15	18	5	4	190
	sw × sd pm	115	103	50	40	56	46	10	4	424

TABLE 18—*Four-point experiments with chromosome-II*

Loci	Type of cross	Non-crossovers	Single crossovers			Double crossovers			Triple crossovers	Total
			Region 1	Region 2	Region 3	1,2	1,3	2,3		
dh b py sd.............	dh b py sd × +	... 68	... 47	... 25	... 0	... 9	... 4	... 4	... 0	157
dh b sd pm............	dh b sd pm × +	... 198	... 140	... 115	... 62	... 54	... 35	... 16	... 7	627
	dh b × sd pm	... 32	... 23	... 23	... 12	... 14	... 0	... 5	... 7	116
b a-w py pm...........	b py pm × a-w	139 ...	... 14	62 ...	71 ...	... 4	... 5	23 ...	... 3	321
b S-a sd pm...........	b sd pm × S-a	96 ...	... 22	42 ...	50 ...	... 12	... 15	4 ...	... 1	242
b sw sd pm............	b sd pm × sw	50 29	22 8	15 13	18 11	10 5	8 8	8 1	2 0	208
b py sd pm............	b py sd pm × +	214 191	84 109	20 24	78 75	4 9	25 24	0 3	0 2	862
	b sd pm × py	178 154	102 82	11 18	60 58	4 6	38 27	0 1	0 0	739

TABLE 19—*Five-point experiment with chromosome-II (Truncate offspring only)*

Loci	Type of cross	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	1,3,4	Total
T b py sd pm...	T × b py sd pm	160	111	105	9	67	59	10	47	4	41	1	3	14	1	632

TABLE 20—*Two-point experiments with chromosome-III*

Loci	Type of cross	Non-crossovers		Crossovers		Total
cu Δ	cu × Δ	...	263	...	182	445
se Δ	se × Δ	62	73	56	54	245
d-l st	d-l st × +	92	48	9	8	157
	d-l × st	38	16	5	5	64
d-l p	d-l p × +	356	317	265	277	1,215
	d-l × p	290	252	241	181	964
st Δ	st × Δ	99	83	49	22	253
st rg	st rg × +	99	73	69	58	299
	st × rg	186	147	137	149	619
H Δ	H × Δ	432	411	12	21	876
Δ bp	Δ × bp	344	311	53	59	767
Δ p	Δ p × +	181	209	133	137	660
	Δ × p	40	45	34	42	161
p rg	p × rg	234	217	4	5	460

TABLE 21—*Three-point experiments with chromosome-III*

Loci	Type of cross	Non-crossovers		Single crossovers				Double cross-overs		Total
				Region 1		Region 2				
cu Δ p	cu Δ × p	61	48	50	36	30	33	25	23	306
cu p rg	cu p × rg	56	40	66	47	3	6	1	0	219
se rh-b st	se × rh-b st	120	159	41	19	20	34	12	4	409
se st Δ	se Δ × st	121	142	70	44	43	79	21	8	528
rh-b st p	rh-b p × st	205	186	45	31	149	122	23	14	775
d-l st Δ	d-l st × Δ	167	132	21	31	44	19	0	11	425
d-l Δ p	d-l p × Δ	158	157	104	95	104	82	59	41	800
st H ap	st H × ap	64	45	13	20	12	12	1	3	170
	st ap × H	83	35	10	11	12	14	6	18	189
st H p	st p × H	251	278	51	69	141	139	26	27	982
st Δ ap	st ap × Δ	190	166	71	69	19	32	12	4	563
	st × Δ ap	88	77	20	29	5	5	2	2	228
st Δ p	st Δ p × +	148	167	67	67	102	74	31	36	692
	st p × Δ	266	228	100	70	139	144	59	49	1,055
	st × Δ p	180	202	78	53	113	91	40	30	787
st Δ ca	st ca × Δ	35	34	9	9	32	26	6	4	155
st p rg	st rg × p	72	79	54	55	0	1	1	1	263
st p M	st p × M	103	...	...	59	...	21	24	..	207
H ap p	H × ap p	78	88	35	19	29	33	9	2	293
Δ bp p	Δ p × bp	134	58	13	21	37	25	0	5	293
Δ p rg	Δ p × rg	199	232	139	123	7	6	0	2	708
Δ p M	Δ M × p	53	...	...	19	...	17	17	..	106
	Δ × p M	...	87	69	...	68	...	..	20	244

TABLE 22—*Four-point experiments with chromosome-III*

Loci	Type of cross	Non-crossovers		Single crossovers						Double crossovers						Triple cross-overs		Total
				Region 1		Region 2		Region 3		1,2		1,3		2,3				
cu st p rg	cu st rg × p	73	55	48	31	70	54	4	2	32	41	3	3	3	4	0	0	423
cu st Δ p	cu st p × Δ	78	71	47	40	40	29	40	54	13	10	28	21	17	17	4	1	510
	cu Δ × st p	77	67	39	33	32	25	42	37	14	12	25	16	6	11	8	10	454
rh-b st H p	rh-b st p × H	84	67	17	20	25	21	38	37	4	1	8	12	11	10	2	2	359
st H Δ p	st H p × Δ	182	182	47	43	8	2	99	102	1	2	15	20	2	0	0	0	705
	st p × H Δ	320	330	69	75	7	11	181	181	3	0	41	29	3	1	0	1	1,252
st Δ bp p	st bp p × Δ	254	100	53	69	25	47	119	57	10	5	13	29	4	13	2	1	801
st Δ p M	st Δ p M × +	...	126	48	...	77	...	51	...	..	22	..	25	..	28	4	..	381
	st p M × Δ	...	65	19	...	40	...	34	...	..	10	..	7	..	8	4	..	187
	st p × Δ M	141	...	...	43	...	81	...	49	8	..	17	..	26	..	..	6	371
	st × Δ p M	21	...	...	4	...	15	...	5	2	..	3	..	4	..	..	2	56
st Δ ca M	st ca × Δ M	39	...	...	4	...	24	...	0	8	..	0	..	0	..	..	0	75

II

VARIATION IN CROSSING OVER IN RELATION TO AGE OF FEMALE IN DROSOPHILA MELANOGASTER

By Calvin B. Bridges

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VARIATION IN CROSSING OVER IN RELATION TO AGE OF FEMALE IN DROSOPHILA MELANOGASTER

By Calvin B. Bridges

Review of "A Linkage Variation in *Drosophila*," Bridges 1915

The fact that the amount of crossing over may change according to the age of the female was first observed in connection with linkage studies on the second chromosome. In order to obtain a larger sample of offspring from a given female, she was transferred, at the end of her usual 10-day laying period, to another culture bottle and a second 10-day brood was raised. It was found that the second brood from such females showed less recombination of the linked characters than did the first broods from those same mothers. Thus, for black and purple (see table 1) the first broods gave a total of 3,934 flies, of which 212, or 5.4 per cent, were recombinations due to crossing over. In the second broods from the same mothers there were 4.2 per cent of recombinations. The decrease of 1.2 was a 22 per cent decrease from the initial value of 5.4. The greatest percentage decrease was for the section just to the right of the purple, namely, 32 per cent for the purple vestigial data. There was only a 10 per cent decrease for the Streak morula recombination. But, of the total crossing over that occurs between Streak and morula (about 91 cases per hundred gametes), less than half (about 43/91) was detectable through recombination of the characters Streak and morula. The remainder (roughly 48 cases per hundred gametes) consisted of double crossovers (24 cases of double crossing over), and each double crossover leaves undisturbed and in the original combination all genes not included between the two crossovers of the double. Thus, the decrease of 10 per cent in recombination corresponded to a decrease of about 21 per cent in crossing over (91:43::x:10. $x = 21$). For the other examples in table 1 the amounts of double crossing over involved are known

Table 1—*Summary of second-chromosome data from Bridges 1915, showing decrease of crossing over for 10-day second broods*

Section	First broods			Second broods			Change in Rn.	Per cent change in Rn.	Per cent change in C.O.
	Total	Rn.	Per cent Rn.	Total	Rn.	Per cent Rn.			
black to purple...	3934	212	5.4	2693	112	4.2	−1.2	−22.	−22.
black to curved...	6271	1439	22.9	4712	897	19.0	−3.9	−17.	−19.
purple to vestigial.	5174	608	11.8	5415	431	8.0	−3.8	−32.	−32.
purple to curved..	3934	711	18.1	2693	382	14.2	−3.9	−22.	−23.
Streak to morula..	168	71	42.3	131	50	38.1	−4.2	−10.	−21.

to be relatively small, and accordingly the percentages of recombination are more direct indices of the changes in crossing over.

In one of the experiments, involving black and curved (table 5, Bridges 1915), three and four 10-day broods were raised. These indicated that the fall reached a minimum in the second broods, and thereafter crossing over rose nearly to the initial value.

In another of the experiments (table 7, Bridges 1915), three loci, black, purple and curved, were used simultaneously. In the discussion of this experiment two variables were distinguished: first, change in the relative ease of occurrence of crossing over in a given region; and second, change in the relative spacing of the crossovers that do occur. These two factors have been called "coefficient of crossing over" and "internode length." It was seen that changes in the internode length should affect the proportionate number of double crossovers. The relative frequency of double crossovers was studied by means of the "coincidence" index, which is the ratio of the observed percentage of doubles to the percentage expected on random distribution. The coincidence index is the reciprocal of the earlier "interference" index of Muller and has proved more generally useful. The data were meager but suggested that the average internode had changed concomitantly with the change in crossing over.

Data upon first and second broods for sex-linked characters showed no change in crossing over. The sections followed were white to sable, white to forked, vermilion to Bar, vermilion to fused, and Bar to fused. These sections covered practically all of the X-chromosome known at that time, the mutant bobbed, still further to the right than fused, not yet having been found.

The only third-chromosome case studied was that of pink and kidney, which was unsatisfactory because of the uncertainty in classifying kidney. The data indicated a slight rise for second broods. When the calculations are made on the basis of the kidney flies only, a more reliable method, the rise is not as great. Later work (see below) has shown that a rise for this region of the third chromosome is very atypical and it is possible that the rise indicated was entirely due to error.

Review of Data on Relation Between Age of Mother and Frequencies of Crossing Over, from "The Effect of Temperature on Crossing Over in *Drosophila*," Plough 1917.

In the course of his tests of the effect of differences in temperature upon crossing over in the second chromosome, Plough secured numerous data, in the nature of controls, upon the relation of crossing over to the age of the mother.

Table 2 gives a summary of the data from first and second broods for the backcrosses of Star by black curved and of black purple curved by wild (table 11, Plough 1917). The use of Star provided information as to the change for the left half of chromosome-II, a region not adequately investigated by Bridges. Plough's data gave a greater change for black purple (70 versus 22) and a less change for purple curved (9 versus 23) than did the corresponding data of Bridges.

In table 3 the data from both papers are combined. The greatest change was for black purple and for purple vestigial, which represent the mid-region of the chromosome. The regions to the left and to the right of the mid-region were somewhat less subject to change.

TABLE 2—*Summary of second-chromosome data from Plough 1917, showing decrease of crossing over for 10-day second broods*

Section	First broods			Second broods			Change in Rn.	Per cent change in Rn.	Per cent change in C.O.
	Total	Rn.	Per cent Rn.	Total	Rn.	Per cent Rn.			
Star to black.....	1195	432	36.1	1331	409	30.5	−6.4	−18.	−22.
Star to curved....	1195	555	46.5	1331	532	39.9	−6.6	−14.	−22.
black to purple...	2219	132	5.9	966	18	1.8	−4.1	−70.	−70.
black to curved...	3414	762	22.3	2297	386	16.8	−5.5	−25.	−27.
purple to curved..	2219	433	19.5	966	173	17.9	−1.6	− 8.	− 9.

In the black purple curved backcross Plough raised third broods, and these gave values higher than those of the second broods but lower than those of the firsts, as was also the case in the data of Bridges.

Plough made a decided improvement in the technique of studying the effect of age upon crossing over in that he divided the egg-laying into short periods, usually of two days. Thus, in tables 13 and 14 (Plough 1917) he gave data from two experiments in which females heterozygous for three recessives, black, purple and curved, were mated to black purple curved males and were allowed to lay eggs in a succession of two-day periods. In figures 1 and 2 are given graphs of the change in crossing over with progressive age of the mothers. The different parts of the curves do not have equal reliability, since during the course of the experiments the number of mothers became reduced by death and sterility, and also the productivity of those remaining decreased. Thus, the first 20 days of the graphs of figure 2 are based on about 1,000 flies per two-day period, while from 25 days on, the number was less than 200. The first 20 days of the graphs of figure 1 are based on about 400 flies per period and the remainder are about 200

TABLE 3—*Summary of tables 1 and 2*

Section	First broods			Second broods			Change in Rn.	Per cent change in Rn.	Per cent change in C.O.
	Total	Rn.	Per cent Rn.	Total	Rn.	Per cent Rn.			
Star to black.....	1195	432	36.1	1331	409	30.5	−6.4	−18.	−22.
Star to curved....	1195	555	46.5	1331	532	39.9	−6.6	−14.	−22.
Streak to morula..	168	71	42.3	131	50	38.1	−4.2	−10.	−21.
black to purple...	6153	344	5.6	3659	130	3.6	−2.0	−36.	−36.
black to curved...	9685	2201	22.7	7009	1283	18.3	−4.4	−19.	−21.
purple to vestigial.	5174	608	11.8	5415	431	8.0	−3.8	−32.	−32.
purple to curved..	6153	1144	18.6	3659	555	15.2	−3.4	−18.	−19.

flies per period. In figure 2 the smoothed curve for black purple recombination showed an initial high value (8.2), a slow fall to a minimum (4.6) at about the 13th day, a fairly rapid rise to a second high value (8.0) at the 20th day. A second fall reached, at the 30th day, a level (3.0) that was maintained with little change to the end of the experiment at the 38th day. The smoothed black purple curve of figure 1 showed about the same relations as that of the more reliable curve of figure 2, but the whole curve is compressed into shorter time. The initial fall was rapid, reaching a minimum at the 6th day. The succeeding rise was slow, reaching a maximum at about the 17th day. The second fall reached a level at the 22d day and this level was maintained to the end of the experiment at the 33d day. For both experiments the curves for purple curved were not greatly different from those for black purple, but were proportionately of lesser magnitude of change.

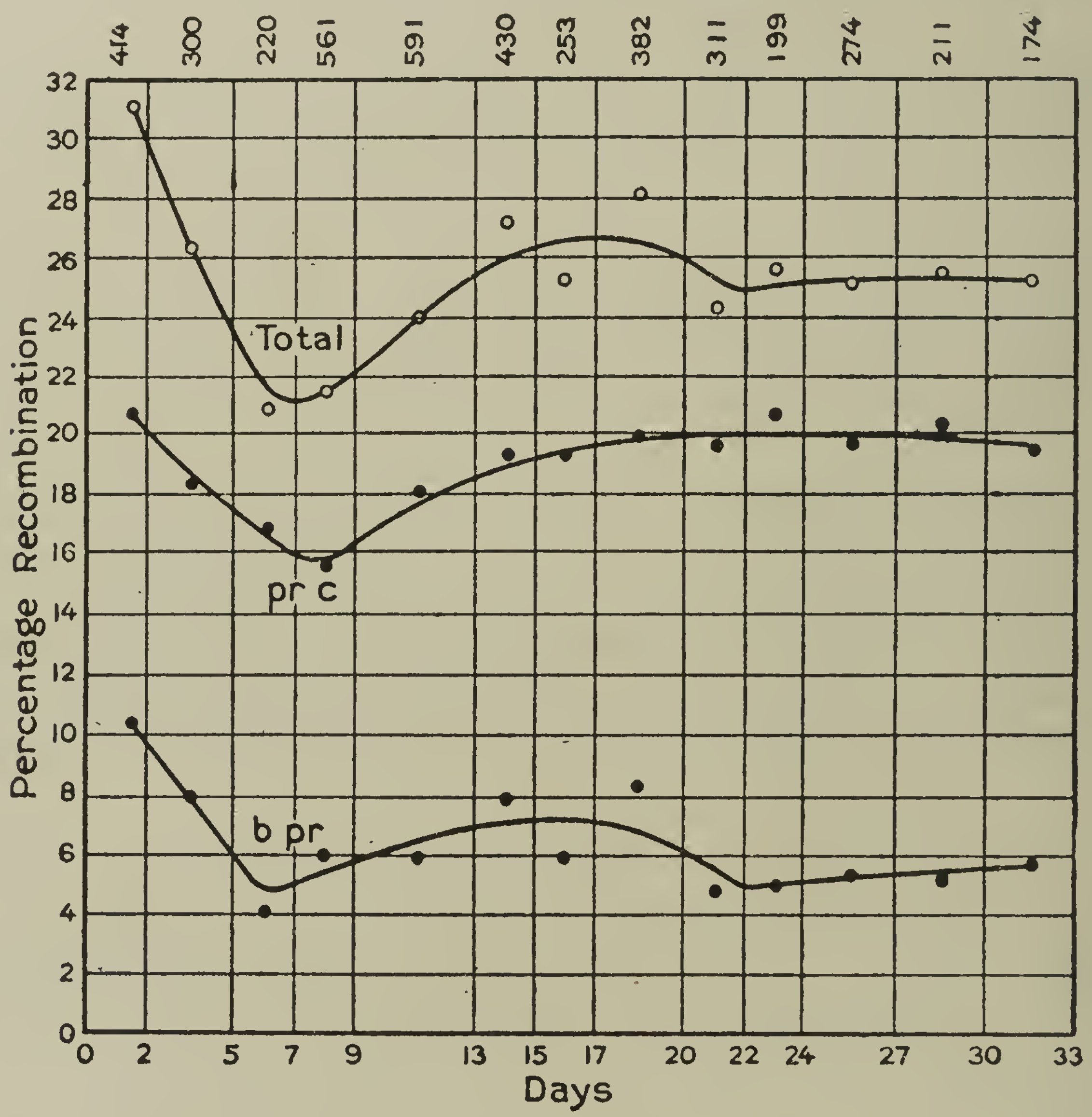

Fig. 1.

Review of the Data on Effect of Age Upon Crossing Over, from "Further Studies on the Effect of Temperature on Crossing Over," Plough 1921.

Plough tested the effect of differences in temperature and in age upon the X-chromosome by means of backcrosses with a multiple stock "Xple" provided by Bridges and consisting of the recessives scute, echinus, cut, vermilion, garnet and forked. As control for the temperature test, and as a test of the age effect, certain backcross parents were transferred every three or four days to fresh culture bottles which were kept at 24°C. The graphs for the amount of crossing over with progressive age showed no significant change in any of the sections followed (table 1 and figure 1, Plough 1921). Other backcross parents were raised at 31.5°C, but the matings and transfers were carried out as before at 24°C. The first offspring from the heat-treated parents showed the same values as did the control, and there was no change with age. The lack of effect of age upon crossing over in the X-chromosome (scute to forked) was in agreement with the earlier findings by Bridges (1915) and by Plough (1917).

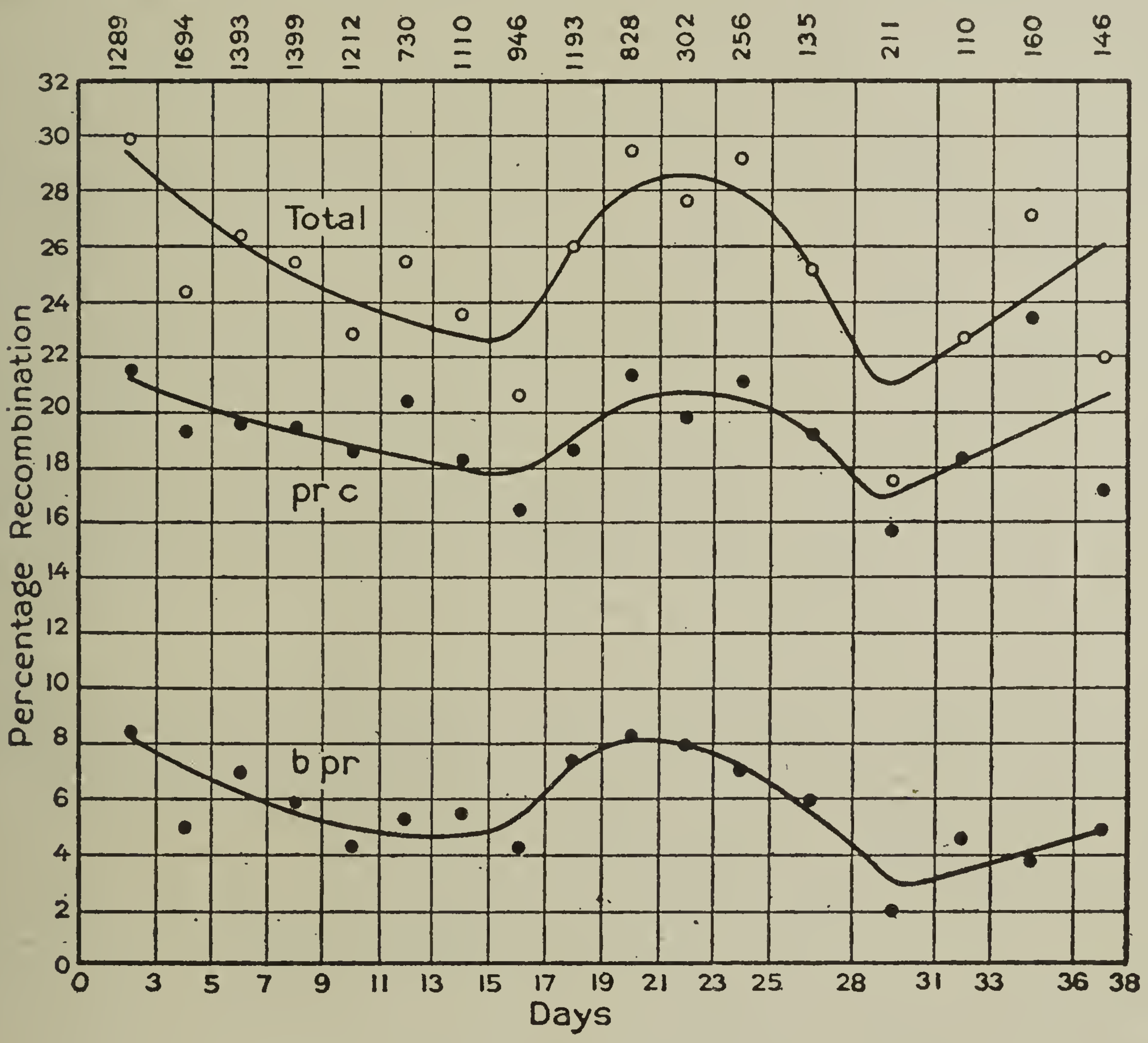

Fig. 2

Plough applied the same method of two-day laying periods in studying the effects of differences in age and temperature on the third chromosome. He found that the sections from sepia to Dichæte and from Dichæte to spineless showed marked effects of temperature changes and somewhat less effects of age differences. The sections from spineless to sooty and from sooty to rough showed only slight change with either age or temperature (tables 2, 3, and figure 2, Plough 1921).

Full Account of the Experiments Dealt with in "The Relation of the Age of the Female to Crossing Over in the Third Chromosome of *Drosophila Melanogaster*," Bridges 1927.

The Stocks IIIple and Alternated IIIple

Stocks satisfactory for any precise study of the effects of age upon crossing over in the third chromosome were not in existence, nor were enough workable mutant characters available to construct them, until 1919. The most useful multiple stocks on hand previously were sepia spineless sooty rough and peach spineless sooty rough, both assembled by H. J. Muller. Sepia and peach could not be used together. The dominant Dichæte, found by Bridges, July 1915, could be used with either of these multiple stocks. In 1916, scarlet was found by M. H. Richards and by D. E. Lancefield and, on the basis of backcrosses with Dichæte and with other mutants, it was supposed that its locus was to the left of Dichæte by about three units. The double recessive scarlet peach was found to be a light orange eye-color easily distinguishable from both scarlet and peach. The discovery of hairy by O. L. Mohr, December 1918, and the determination of its locus at half a unit to the right of sepia gave a substitute for sepia in a character that could be used with any eye-color. Finally, roughoid was found by A. H. Sturtevant, February 1919, and was located at some 26 units to the left of hairy at a new left end of the third chromosome. However, roughoid and rough could not be used together with entire success.

The mutants roughoid, hairy, scarlet, Dichæte, peach, spineless and sooty could be used together satisfactorily to cover the left end and the middle of the third chromosome. It was decided to utilize these mutants without waiting for others to arise in the right end of such a nature that they could be used with those already on hand and thereby cover the entire third chromosome.

For the best use of several mutants simultaneously in linkage experiments three stocks were required, namely, the multiple recessive and two stocks which give, when crossed, the multiple heterozygote with alternated placement of the mutants. The multiple recessive required in this case was roughoid hairy scarlet peach spineless sooty, a stock that has been called "IIIple." The two stocks for the alternated heterozygote were respectively roughoid scarlet peach sooty and hairy Dichæte spineless. The alternated backcross which would furnish the data of the experiment would then be between the multiple heterozygotous F_1 female $\left(\frac{ru \quad\quad st \quad\quad p^p \quad\quad e^s}{\quad h \quad\quad D \quad\quad ss \quad}\right)$ and the multiple homozygous recessive male (ru h st p^p ss e^s). The special advantage of such an alternated backcross is that it distributes the mutant characters with a maximum of uniformity to the flies that appear, nearly all of which receive either three or four mutant characters. Where there is little difference in the viability of the mutants, as is the case with the seven mentioned, the lowering of the viability of a fly is the greater the higher the number of mutants the fly carries, and it makes relatively little difference which mutants these are. For an alternated backcross the inviability effects are distributed uniformly and the classes that appear are more nearly proportional to the gametic classes than with any other type of backcross.

First Experiment

The three stocks described above were under preparation during 1920, and an experiment was started as soon as the alternated stocks were secured in January 1921. Twelve heterozygous females were mated to multiple recessive males, and each was allowed to remain in a 4-inch × 1-inch flatbottomed culture vial for two days. Each was then removed to a fresh vial for two more days, and this procedure was continued as long as any female remained alive. As the flies hatched from day to day in each of these culture vials, they were classified, recorded and finally the totals for each class were found for each vial.

As soon as the backcross flies began to emerge in the tubes it became apparent that the results differed in two respects from expectation. In the first place, the mutant hairy was not present at all. It is probable that hairy was lost through unexpected crossing over during the later stages of preparing the h D ss stock. In the second place, the locus of scarlet was found to be, not to the left of Dichæte as supposed, but to the right, between Dichæte and peach. The orientation of the mutants in the multiple heterozygote was therefore $\frac{\mathrm{ru}\qquad\qquad\qquad\mathrm{st}\quad \mathrm{p^p}\qquad\qquad \mathrm{e^s}}{(1)\ \ \mathrm{D}\ \ (2)\ \ (3)\ \ (4)\ \ \mathrm{ss}\ \ (5)}$. While this orientation was not as satisfactory as the one planned, yet it was decided to complete the counts and later to carry out a strictly alternated experiment. The two experiments would supplement and check each other.

The completed total counts for the separate two-day cultures were tabled, according to parentage, and all two-day cultures having the same mother (*e. g.,* the offspring of female No. 3) were added together to give the total progeny per pair.

The twelve progenies were then compared with one another to determine whether they were similar beyond limits that were significant. It was apparent upon simple inspection that the progenies were of two very distinct types. Seven were of the expected type, while five differed in that the output of flies was only about a twelfth as great as normal, and in that the crossing over was also very low, the total recombination being about a third that given by the others. The five similarly aberrant progenies were separated out and will be treated later (pages 82–86).

The remaining seven progenies were further analyzed and compared, as follows: The two separate complementary classes that went to make up each class with respect to recombination were compared to detect any significant departures from equality. No cases were found in which the inequality was unexpectedly large, and in the totals for the entire seven progenies the complementary classes were in good agreement throughout, as is evident from the samples given: 0, ru st $\mathrm{p^p}$ $\mathrm{e^s}$ = 2247, D ss = 2180; 1, ru D ss = 1132, st $\mathrm{p^p}$ $\mathrm{e^s}$ = 1090; 2, ru ss = 35, D st $\mathrm{p^p}$ $\mathrm{e^s}$ = 45; 3, ru st ss = 100, D $\mathrm{p^p}$ $\mathrm{e^s}$ = 113; 4, ru st $\mathrm{p^p}$ ss = 230, D $\mathrm{e^s}$ = 225; 5, ru st $\mathrm{p^p}$ = 329, D ss $\mathrm{e^s}$ = 312. From this comparison it was evident that the distribution of mortality had been substantially uniform and that it was therefore legitimate to add together the complementary classes and deal thereafter with a single class that represented the total with respect to each kind of combination or recombination. This was done, and the simplified progenies were then reexamined more critically as to uniformity in the crossing-over relations. No significant or unusual differences between the seven progenies were discovered. The amounts of recombination were fairly uniform both in total and in distribution for all five sections of the chromosome for which crossing over was being examined.

The various tube totals for these seven progenies were then resummarized, by adding together all tubes having the same age of the parents, *e. g.*, all seven tubes in which the parents were present the first two days after mating, or the 11th and 12th days after mating, etc. This summary is given in table 4.

TABLE 4—*Experiment 1. Offspring of 7 females* $\left(\frac{ru \quad + \quad st \quad p^p \quad + \quad e^s}{+ \quad (1) \quad D \ (2) + (3) + (4) \ ss \ (5) +}\right)$, *fertilized by ru h st* p^p *ss* e^s *males. Data summarized according to age of mothers*

Age period	0	1	2	3	4	5	1 2	1 3	1 4	1 5	2 3	2 4	2 5	3 4	3 5	4 5	1 2 3	1 2 4	1 2 5	1 3 4	1 3 5	1 4 5	2 4 5	1 2 4 5	1 3 4 5	Total
0– 2...	135	77	4	10	21	27	2	5	15	22	1	..	..	2	1	2	..	..	1	1	2	2	..	..	1	331
2– 4...	391	250	7	24	68	89	2	14	30	53	..	1	1	2	6	5	..	1	..	1	2	1	..	..	1	949
4– 6...	524	295	12	18	86	88	2	9	42	48	..	5	1	2	6	1	..	..	..	1	..	2	..	..	..	1142
6– 8...	584	291	7	13	56	91	..	9	35	61	..	1	2	1	1	2	..	..	..	2	..	1	..	..	..	1157
8–10...	648	298	7	6	33	77	1	1	13	48	..	..	2	..	1	3	..	..	..	..	..	1	..	..	..	1139
10–12...	510	204	9	16	41	60	1	4	13	34	..	..	1	4	1	5	..	..	1	1	1	..	..	..	..	906
12–14...	549	274	9	23	35	62	3	10	15	36	1	..	1	1	3	3	..	..	..	..	1	1	..	..	1	1028
14–16...	394	200	8	13	32	54	..	3	18	30	..	..	1	1	2	3	..	..	..	2	2	..	..	..	..	763
16–18...	327	155	7	27	34	46	..	8	18	16	..	2	..	4	3	3	..	1	..	..	1	..	..	..	..	652
18–20...	153	87	3	17	17	23	..	7	10	11	..	..	..	2	3	4	1	..	..	4	1	..	..	1	..	344
20–23...	131	69	3	26	16	13	2	12	9	3	..	1	..	1	2	4	..	..	..	..	..	..	1	..	..	293
23–25...	29	10	2	6	5	5	1	3	2	2	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	66
25–27...	23	8	..	7	7	4	..	1	..	2	..	..	..	..	1	..	..	..	..	1	..	..	..	..	..	54
27–29...	29	4	2	7	4	2	1	..	2	3	..	..	..	2	..	..	..	..	..	1	2	..	..	..	..	59

[1] The data of table 4 are different from the raw data by two processes of summation and by the elimination of five pogenies. The raw data and the first summation are not given since they form tables that are very bulky. These tables are held on file and are available for anyone who desires to analyze the data further.

From the data of table 4, a new table was prepared that gave for each of the five crossing-over sections the total number and the percentage of recombinations that occurred for each two-day age class. The values of this table (which also is on file but not published) are plotted in figure 3, which relates graphically the age of the mother (abscissæ) with the percentages of recombination shown by the offspring (ordinates). For each of the crossing-over sections the successive points were connected by light lines and a smooth curve was drawn freehand. An additional series of points with a smoothed curve represents the total recombination, which is the sum of the five separate percentages of recombination. The scale for this last curve is given by the numbers (50, 60, 70, 80) just inside the left margin.

The reliability of the different parts of these curves varies greatly. In the first place, the total flies produced in the various two-day periods differed greatly, as can be seen from the totals given for each two-day period at the top of the figures. A customary statistical index of reliability is the reciprocal of the standard error: $1/\sqrt{p\ q\ /\ N}$ or $\sqrt{N\ /\ p\ q}$. That is, the reliability of the points in figure 3 varies with the square root of the number of individuals on which the determinations are based. In the second place, the total number of mothers (7) remained alive only through the 16th day, and accordingly the succeeding lots of flies are from a parentage that is less and less directly comparable with that of the first 16 days. In the third place, it is probable that different females died at different times, partly because they aged at different rates. Accordingly, the diversity in physiological age became increasingly great as the flies approached the point at which they began to die, and the results are correspondingly less reliable. From all these considerations it is probable that the most reliable part of the system of curves lies between the third and fourteenth days.

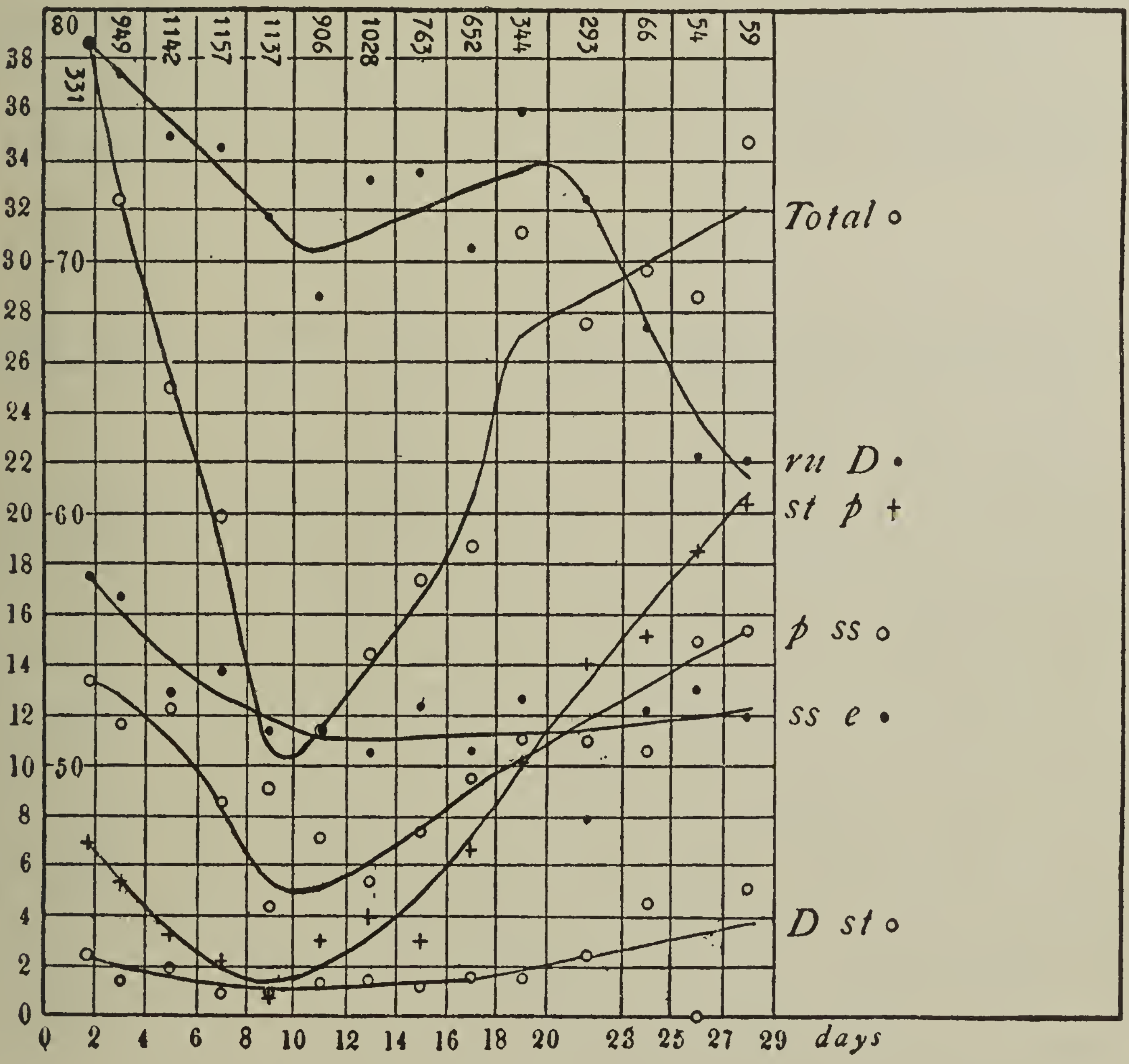

FIG. 3

Inspection of the curves shows that in total recombination there was an initial high value (78.7) followed by a rapid nearly straight-line decrease to a minimum value (50.3) at about the tenth day. There was then a sharp inflection with a slower nearly straight-line rise to the 17th day. Beyond the 17th day there was a sudden rise to a high level that was maintained. Each of the separate recombination curves likewise shows an initial high value and a gradual decrease. The minimum value was reached at only slightly different times by the different curves. The proportionate fall from the initial high value to the first minimum value is markedly different for the different sections, as shown below.

Section	Decrease	Per cent change
st to p^p	6.8 to 1.3 = 5.5	81
p^p to ss	13.3 to 5.0 = 8.3	62
D to st	2.3 to 1.1 = 1.2	52
ss to e^s	17.5 to 11.0 = 6.5	37
ru to D	38.7 to 30.5 = 8.2	21
Total	78.7 to 50.3 = 28.4	36

Following the minimum value, each curve, except that for ss e^s, underwent a rise to a greater or less extent. Beyond 16 days the courses of the curves became increasingly diversified, probably with little significance. A striking appearance was made by the st p^p curve, which mounted from a low of 1.3 to a high of 21 at the end of the experiment.

Second Experiment

The discovery that the locus of scarlet is to the right of Dichæte necessitated that the P_1 stocks be recast to give the following heterozygote: $\frac{ru \qquad D \qquad p^p \qquad e^s}{(1)\ h\ (2)\ (3)\ st\ (4)\ (5)\ ss\ (6)}$. The two new stocks ru D p^p e^s and h st ss were ready in March 1921 and a second backcross experiment was immediately carried out in two-day culture tubes as the first had been. Two slight changes in procedure were made: Since it was observed that females ordinarily lay no eggs during the first day after emergence and mating, and only about two-thirds the normal number on the second day, the pairs were left in the first vial three days and in each subsequent vial two days. The experience of Clausen (1924) with two-day vials had shown that while the output in number of flies per pair was relatively larger, the ratios (especially with respect to vestigial) tended to be very aberrant. Also the flies produced were small and pale in color. These facts suggested that conditions in the vials had encouraged abundant egg-laying but that the amount of food used was insufficient for the proper development of all the larvæ. In the first experiment the amount of food used was approximately double that used by Clausen. In the second experiment, the amount was still further increased, for, although the ratios were not aberrant, the flies, especially the last to emerge, were somewhat small and pale. The vials were filled from an inch and a quarter to an inch and a half deep with the banana-agar media (Bridges 1921) and were then allowed to set in a slanted position so that the bottom was exposed for a tiny segment. This bare segment was for the escape of CO_2, which would otherwise accumulate behind the medium and force it out of place. On the slant surface were placed a few drops of yeast suspension and about four thicknesses of towel paper cut 1″ × 2.5″. The culture vials were kept in a 25°C incubator in racks which were set at the same angle as the slant of the food medium, and which maintained the surface of the medium horizontally.

It may be noted here that experience since this experiment was carried out shows that even this amount of food is below optimum. It is suggested that additions of compressed live yeast be made to the vials to supply the extra food needed.

The parents in the second experiment lived longer than those in the first. One of the eight females died on the 15th day, but none other died until the 29th day. The last three females died on the 39th day.

As in the first experiment, the separate tube results were tabled and totaled according to parentage. The eight progenies were seemingly of a single type, and a rather detailed comparison of the amounts of recombination due to crossing over in the six different sections did not bring to light any differences of significant magnitude between them. The curves for the changes in amount of recombination were likewise substantially the same for the eight cultures.

TABLE 5—*Experiment 2. Offspring of 8 females* $\left(\frac{ru}{+}\;(1)\;\frac{+}{h}\;(2)\;\frac{D}{+}\;(3)\;\frac{+}{st}\;(4)\;\frac{p^p}{+}\;(5)\;\frac{+}{ss}\;(6)\;\frac{e^s}{+}\right)$, *fertilized by ru h st p^p ss e^s males. Data summarized according to age of mothers*

Age period	0	1	2	3	4	5	6	1 2	1 3	1 4	1 5	1 6	2 3	2 4	2 5	2 6	3 5	3 6	4 5	4 6	5 6	1 2 4	1 2 5	1 2 6	1 3 6	1 4 5	1 4 6	1 5 6	2 3 5	2 4 5	2 4 6	2 5 6	3 5 6	1 2 4 5	1 2 5 6	2 3 4 6	Total
0– 3..	312	122	61	10	17	53	60	6	.	11	26	14	1	4	5	10	1	.	3	5	3	1	5	.	.	.	.	2	.	.	.	.	.	.	.	.	732
3– 5..	778	298	165	16	30	92	131	9	6	13	38	52	.	1	22	18	.	1	3	4	3	.	.	.	1	1	3	2	.	1	.	3	1	.	.	.	1692
5– 7..	713	250	131	15	13	64	113	6	3	3	26	37	1	1	11	22	1	2	4	1	4	.	1	2	.	1	1	.	.	.	.	1	.	.	.	.	1427
7– 9..	716	228	92	6	4	54	110	5	1	1	13	32	.	1	4	8	3	2	1	.	3	.	.	.	.	.	.	.	1	.	.	.	.	1	.	.	1286
9–11..	790	249	111	8	8	57	84	3	2	2	7	16	.	.	10	8	1	1	.	.	1	.	1	3	.	.	.	.	.	.	.	.	.	.	.	1	1363
11–13..	769	260	85	5	5	39	79	3	2	2	13	26	.	.	3	11	.	.	.	1	1	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1305
13–15..	673	207	97	6	11	40	86	4	2	2	6	30	.	.	3	11	1	.	.	.	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	.	1181
15–17..	550	187	79	5	10	32	59	6	.	2	9	27	.	.	5	10	.	.	1	.	2	.	.	.	.	.	.	1	.	.	.	.	.	.	1	.	986
17–19..	512	170	70	7	7	33	66	4	1	1	5	25	.	.	3	16	1	2	.	1	.	.	.	1	.	1	.	.	.	.	.	.	.	.	.	.	926
19–21..	383	121	45	7	7	15	57	5	.	1	5	20	.	1	1	9	.	1	1	.	1	.	.	.	.	1	.	1	.	.	1	.	.	.	.	.	683
21–23..	508	156	75	7	16	19	45	5	1	1	6	17	.	1	2	7	1	.	.	2	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	871
23–25..	512	142	75	6	8	20	64	3	.	1	4	12	.	.	2	7	.	.	.	.	1	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	859
25–27..	346	131	39	2	6	13	51	.	.	..	4	15	.	1	1	9	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	619
27–29..	328	91	40	6	1	20	43	3	1	2	9	10	.	1	1	7	.	.	1	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	566
29–31..	166	53	31	1	3	9	27	1	1	..	2	6	.	1	3	1	1	.	.	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	309
31–33..	149	52	17	2	2	4	21	1	.	..	2	8	.	1	1	1	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	263
33–35..	98	28	10	2	3	5	6	.	.	..	..	8	.	.	3	2	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	166
35–37..	54	24	7	..	1	3	8	1	.	..	..	4	.	.	..	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	106
37–39..	116	32	22	..	..	7	15	.	.	1	1	6	.	.	..	..	.	.	.	1	.	.	1	.	.	1	.	.	.	.	1	.	.	.	.	.	204

As anticipated, the alternated backcross method resulted in a very uniform distribution of the mortality. This can be most easily seen in the larger classes, some of which are given: **0**, ru D p^p e^s = 4234, h st ss = 4239; **1**, ru h st ss = 1374, D p^p e^s = 1426; **2**, ru st ss = 650, h D p^p e^s = 602; **3**, ru D st ss = 56, h p^p e^s = 55; **4**, ru D ss = 75, h st p^p e^s = 77; **5**, ru D p^p ss = 271, h st e^s = 308; **6**, ru D p^p = 568, h st ss e^s = 557.

The results of the above analyses made it possible to combine complementary classes throughout, and to combine the eight progenies into totals that could be treated as a single body of data. Accordingly, the separate tube cultures were resummarized by adding all that came from parents of a given age class. The totals for this summary are given in table 5.

From the results in table 5, a new table was prepared that gave for each crossing-over section the total number and percentage of the recombinations that occurred for each two-day period. These values were plotted (figure 4) and the curves were smoothed freehand. A curve for the total recombination was added, with a special scale just inside the left margin. For the sake of comparison with the first experiment, a curve of the recombination for the roughoid Dichæte interval has been included.

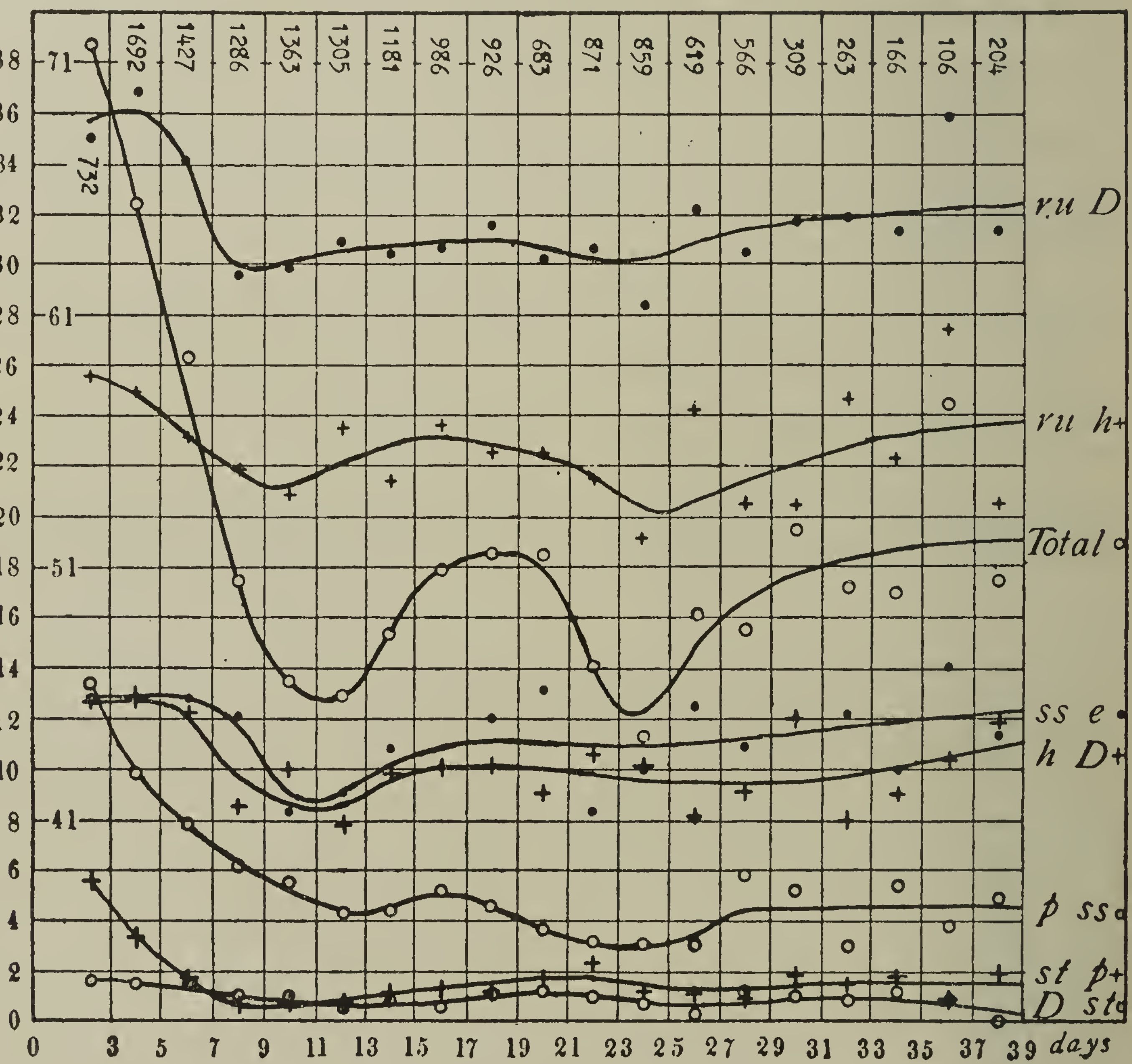

FIG. 4

The reliability of the curves of the second experiment is greater than that of the first; for the distribution of mortality was more uniform; an additional locus (hairy) was followed; eight instead of seven mothers were used; these mothers lived longer; and the productivity was greater (averaging 1,950 per female). The most reliable section of the curves is that included between the third and the 20th days.

Inspection of the smoothed curves shows that the sum of the separate percentages of recombination started high (71.7) and declined rapidly to a minimum of 45.7 at eleven days. A second maximum was reached at about the nineteenth day. The subsequent sharp dip and recovery in the curve was followed by a long period of rise of slight amount.

Of the separate curves of recombination, those for ru h, D st, st p^p and p^p ss showed courses similar in form to the curve of total recombination, but differed from it and from each other slightly in the times at which the inflections occurred, and markedly in the relative amounts of change.

Two curves, h D and ss e (and also ru D) differed from the others in that the first drop did not begin until the fifth or sixth day, but was then more rapid and reached the minimum as soon as the other curves.

In amount of decrease, expressed as the percentage difference between the initial high and the first minimum, the order of the sections was as follows:

Section	Second experiment		First experiment	Average decrease
		per cent	*per cent*	*per cent*
st p^p	5.6 to 0.5 = 5.1	91	81	86
p^p ss	13.4 to 4.2 = 9.2	69	62	66
D st	1.7 to 0.6 = 1.1	65	52	59
Total	71.7 to 45.7 = 26.0	36	36	36
h D	12.7 to 8.4 = 4.3	34	..	34
ss e^s	12.8 to 8.8 = 4.0	31	37	34
ru D	36.1 to 29.8 = 6.3	17	21	19
ru h	25.6 to 21.2 = 4.4	17	..	17

The seriation in the second experiment was the same as in the first, and the values agreed well. The significant feature of this seriation is that the greatest change occurred at the mid-region of the chromosome. There are reasons for believing that the attachment of the spindle fiber is between the loci for scarlet and peach, and it was this particular section that gave the greatest change (86 per cent). The sections immediately to the right of the mid-section (*viz.,* p^p ss) and to the left (*viz.,* D st) were also affected greatly and to about the same extent (66 and 59 per cent, respectively). The second sections (ss e^s to the right and h D to the left) were affected to the same extent but only about half as much (34 and 34 per cent) as were the first sections. Finally, the section farthest removed (ru h) showed least change (17 per cent).

It should also be noted that the curves for recombination change in the symetrically placed second sections (h D and ss e) paralleled each other strikingly and differed in their form from the others (figure 4).

The curves for the percentages of recombination for given sections in experiment 2 were not strikingly similar to those of experiment 1. The curves of experiment 1 were simpler in type as well as of shorter duration. Such a difference had been found by Plough in dealing with the second chromosome, as can be seen from a comparison of figures 1 and 2 of this review.

In the curves for Plough's data can be seen a suggestion of the second minimum and rise that is rather strongly marked in the second experiment with chromosome-III.

It should be noted that the mothers of the different experiments differed in important factors affecting the physiological age. This is apparent in the great difference in length of life and in rate and amount of egg-production.

Multiple Crossing Over, and the Length of the Average Internode

Very early in the *Drosophila* work it was observed (Sturtevant, Muller) that in three-point experiments the number of double crossovers was usually markedly less than the number expected on chance distribution of cross-overs. The deficit of double and of triple crossovers was relatively greater the shorter the total map-distance involved. Evidently, the incidence of one crossover in a given section of chromosome tended to interfere with the occurrence of another crossover in the immediate neighborhood of the first (Muller, 1916). This "interference" has later been commonly expressed by the "coincidence index," which is the ratio of the observed percentage of double crossovers to the percentage expected if crossovers in the first section and simultaneously in any second sections were at random (Bridges, 1915). The easiest way of calculating coincidence is by use of the observed numbers directly, according to the method of Weinstein (1918):

$$\frac{\text{1, 2 doubles} \times \text{Total flies} \times 100}{(\text{1 singles} + \text{1, 2 doubles}) \times (\text{2 singles} + \text{1, 2 doubles})} = C.$$

In constructing maps of the locations of genes in chromosomes (Sturtevant, 1913) the unit of map-distance is that length within which one crossover occurs for every average hundred gametes. There are excellent reasons for supposing that equal intervals on the maps may not correspond to sections of chromosomes of equal length. For example, the high frequency of mutant loci in the neighborhood of pink on the map of the third chromosome, as compared with the low frequency in the region between roughoid and hairy, may be interpreted (Bridges and Morgan, 1923) as a lower frequency of crossing over rather than as a higher frequency of mutation. In the neighborhood of pink it may be supposed there is a low "coefficient of crossing over," and a unit of map-distance represents perhaps five times as long a section of chromosome as does a unit of map-distance in the region to the left of hairy. This uncertainty as to the exact or even relative lengths of chromosome involved makes hazardous any comparison of the ease of double crossing over in different chromosomes, or in different regions of the same chromosome.

Only in case the same section of the same chromosome is involved, is a comparison of the frequencies of simple and multiple crossing over in different experiments free from this uncertainty. In an experiment in which differences in crossing over are compared for different ages of the same mother, not only is the section of chromosome constant but likewise the genetic modifiers of crossing over are constant. The variation is restricted to frequency in occurrence and to variation in spatial distribution of the crossovers within a given section of chromosome. These two types of change have been denominated change in the coefficient of crossing over and change in the average internode (Bridges, 1915). In the first case there is no change in the relative distribution of the crossovers that do occur, while in the last case the proportion of doubles within a given section, and hence the coincidence, changes (Bridges, 1915).

The coincidences for the various combinations of crossing over sections of the second experiment were calculated and plotted. But it was found that the fluctuations were so great that much smoothing was required to reveal the trends of the curves and little reliability was to be placed on the results.

A large source of the variability was the smallness of the percentages of recombination for the sections in the mid-region of the chromosome. In order to reduce this variability the centermost section (st to p^p) and the section immediately to the left (D to st), and to the right (p^p to ss) were combined and were treated as a single section (D to ss). The doubles that were recorded in the original data as occurring within this section from D to ss were counted as two crossovers in the totaling of the crossing over.

Throughout, in totaling the double crossing over, only consecutive crossovers were counted; thus a 1, 2, 3 triple crossover was counted as a 1, 2 double, and as a 2, 3 double but not as a 1, 3 double. This method of treating triple and quadruple crossovers was introduced by Weinstein (1918) and was called "partial coincidence" by Muller (1925). But since it seems to be the method that will be used generally, it is perhaps better to understand by the term "coincidence" this partial coincidence calculated from consecutive crossovers, and to use Muller's term "gross coincidence" in any case in which non-consecutive doubles are included.

The curves for coincidences for the data lumped as outlined above were still not smooth enough to allow much confidence to be placed in the observed trends. Roughly, the curves of coincidence were parallel to those of crossing over, and did not approximate straight, horizontal lines. This fact showed that, in addition to changes in the relative ease of crossing over, there were changes in the relative spacing of the crossovers.

The coincidence index involves the coefficient of crossing over as well as the relative spacing of the crossovers that do occur, for it is calculated from all the flies; *i. e.*, non-crossovers as well as crossovers. But for studying simply changes in the spacing of those crossovers that do occur we may disregard the proportion that they form of the total population. If the average interval between successive crossovers is relatively short, then, of the crossovers that do occur, a relatively large number should be accompanied by a second crossover within a given section, while if the average internode be relatively long, then relatively few associated crossovers should both fall within a given section.

There are several ways of applying this concept to the data in hand. In one method a given section is chosen and the proportion of doubles falling within it is calculated on the basis of all crossovers falling within it. Thus, for the total length followed in experiment 2, namely, from roughoid to sooty, there occurred in the first set of tube cultures a total of 525 crossovers, of which 202, or 38.5 per cent, occurred in multiples. In the data for the second set of tubes, the percentage that the crossovers occurring in multiples was of the total number of crossovers recorded, fell to 33.9, indicating an increase in internode length. The percentages for these and each of the other age-periods are plotted as the large round coördinate points in figure 5, and the smoothed curve is indicated by the W-shaped broken line.

As seen from figure 5, this method of analysis yielded a curve every part of which (except the terminal rise, where the data were meager) was characterized by only slight scattering of the coördinate points and hence by relative certainty as to trend of the curve. The course of the curve shows that the average internode length was varying continually, but with reversals in tendency. During the first ten days the proportionate number of doubles fell, then rose to a maximum at the 19th day, then fell to a second minimum at the 24th day, and thereafter rose to the end of the experiment at the 39th day.

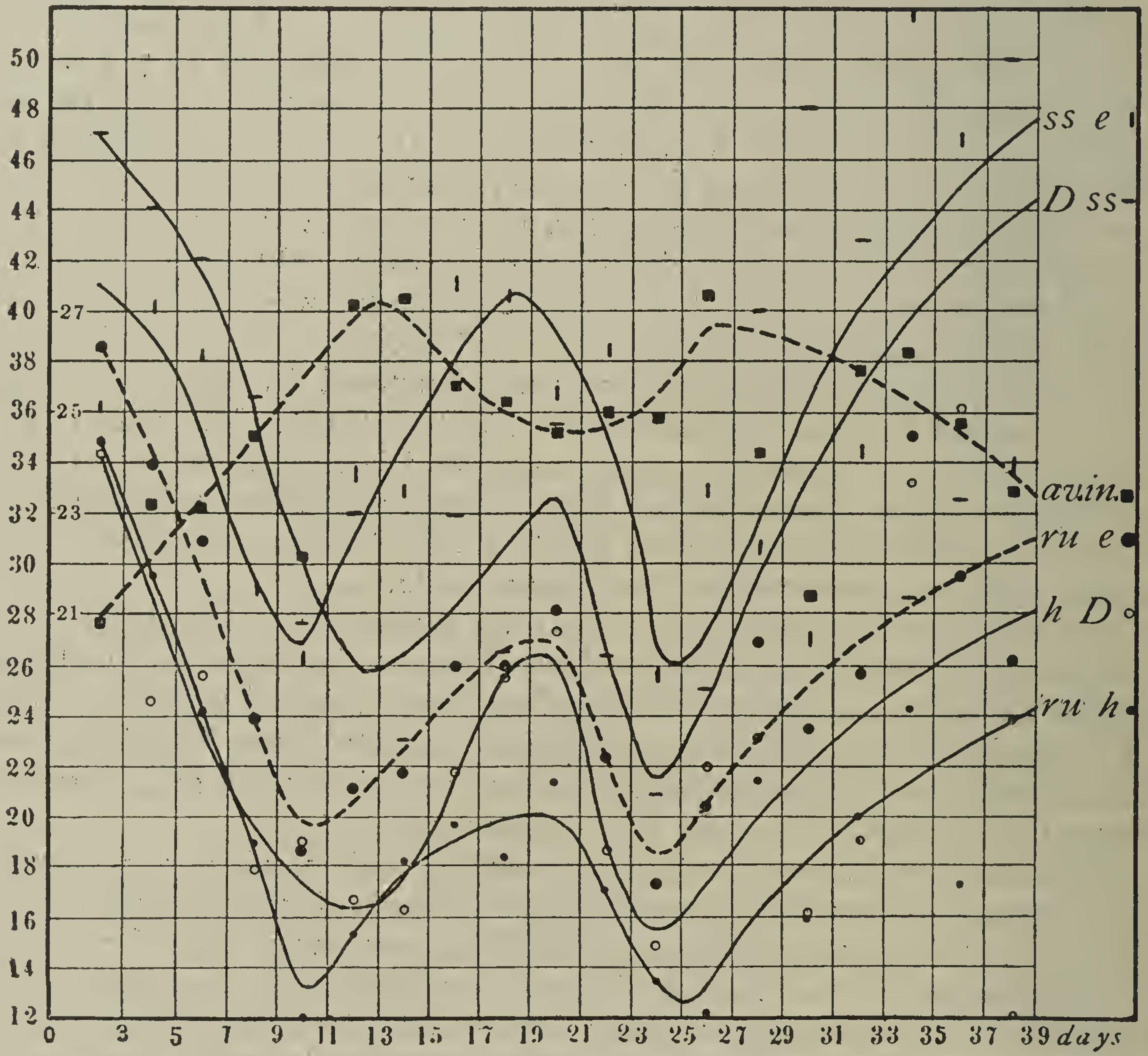

FIG. 5

These changes in relative frequency of multiples were not independent of the changes in total crossing over, for the course of this curve was strikingly parallel, with points of inflection at the same times, as the curve for total recombination given in figure 4. Thus, both the frequencies of crossing over and the average distance between successive crossovers were changing simultaneously. When the frequency of crossing over was low the frequency of crossovers occurring in multiples was disproportionately lower still.

A second method of expressing the relative frequency of multiples in the crossovers that do occur, is by graphs of the percentages of the total crossovers in a given section that are also crossovers in some other section. Thus, for the first set of tubes in experiment 2, there were recorded for section 1 (ru to h) a total of 187 crossovers, of which 122 were simple crossovers and 65, or 34.8 per cent, were coincident with crossing over somewhere else in the chromosome (h to e^s). In the set of tubes whose class center was at the 10th day, there were 281 crossovers, of which 34, or 12 per cent, were associated with crossing over in some other of the sections followed. During this lapse of ten days there was, then, a marked decrease in the relative ease of formation of multiple crossovers in those cases in which one crossover had occurred in section 1. The lowest W-shaped graph of figure 5 (ru h, small solid points) shows for section 1 the changes throughout the experiment in percentage of crossovers occurring in multiples. Similar curves are given for the section from h to D, from D to ss, and from ss to e^s. The curves for these four sections are surprisingly similar to one another, all having the general shape of W, with inflections at practically the same places.

The most direct method of representing the variations in average length of internode, is through calculations of those average lengths in terms of map units. For a basis of comparison of the average lengths given by the separate age groups, the same map units were used throughout, namely, those corresponding to the total of the data of experiment 2. The distances of this map were: 1, ru h = 22.4; 2, h D = 10.3; 3, D st = 1.0; 4, st p^p = 1.7; 5, p^p ss = 5.9; 6, ss e^s = 11.0. The average length of all 1, 2 doubles was taken as half of 22.4 plus half of 10.3, or 16.4. The longest doubles measurable in the experiment were the 1, 6 doubles, which were taken as half 22.4 + 10.3 + 1.0 + 1.7 + 5.9 + half of 11.0, or 35.6. The shortest doubles measurable were the 3, 4 doubles (half of 1.0 + half of 1.7, or 1.4) and the 4, 5 doubles (half of 1.7 + half of 5.9, or 3.8). To find the average internode corresponding to each age period, the observed number of each kind of double was multiplied by the average length of the internode for that double. The sum of these products was divided by the sum of the doubles. Each triple crossover (e. g., 2, 4, 6) was treated as two consecutive doubles (2, 4 and 4, 6) and quadruple crossovers were treated as three consecutive doubles. For the first set of subcultures there were 105 double crossovers and the average distance between the consecutive crossovers was 20.8 units of the special map. For the second set of subcultures there were 194 double crossovers, and the average of the internodes was 23.2 units. The average internode length for each age period is plotted in figure 5 (square coördinate points), and a smoothed curve is drawn (the upper heavy broken line with a special scale just inside the left margin).

The curve of average internode length has roughly the shape of the letter M, and is generally the reciprocal of the other curves of figure 5, which are in terms of ease of formation of multiples. The amount of space necessary for the formation of the average double crossover first increased to a maximum at about the 13th day, then decreased to a minimum at about the 20th day, and thereafter underwent a second rise and fall. The maxima and minima correspond closely to the points of inflection of the W-shaped curve of change in total recombination given in figure 4.

The four methods of expressing the relation of amount of multiple crossing over to age of the mothers agree in showing that the "internode length," or average distance required for double crossing over, has changed in a characteristic fashion, giving an M-shaped curve. These changes have not been independent of changes in total recombination but simultaneous with them. However, the changes in recombination percentages were far greater than could be accounted for by change in internode length and the larger factor must be assumed to be changes in the coefficients of crossing over. The amounts of these changes were greatest for the mid-sections of the chromosomes and least for the distal sections. The changes in the two limbs were of like amount for equal distances from the center of symmetry in the distribution of simple and multiple crossing over.

Aberrant Cultures of Experiment 1; Crossing Over Suppressor C II III

Of the twelve F_1 females used in experiment 1, seven gave the normal type of result seen in table 4, but the other five all gave a new type of aberrant result shown in tables 6 and 7.

TABLE 6—*Offspring of 5 aberrant females from experiment 1* $\left(\frac{ru \qquad + \quad st \quad p^p \quad + \quad e^s}{+ \ (1) \ D \ (2) + (3) + (4) \ ss \ (5) +}\right)$, *fertilized by* *ru h st* p^p *ss* e^s *males*

No.	0		1		2		3		4		5		1,3	1,5	3,4		Total	Total per cent Rn.
	ru st p^p e^s	D ss	ru D ss	st p^p e^s	ru ss	D st p^p e^s	ru st ss	D p^p e^s	ru st p^p ss	D e^s	ru st p^p	D ss e^s	st ss	st p^p	ru st p^p e^s	D ss		
2	53	54	4	3	..	..	5	4	1	3	..	2	1	..	..	1	131	19.8
4	53	41	1	1	..	3	2	4	..	1	2	..	1	..	1	..	110	16.4
8	22	33	3	2	1	..	2	4	..	1	..	1	..	..	..	..	69	20.3
9	63	59	1	3	2	1	2	5	4	3	2	1	..	1	..	..	147	17.7
14	28	39	3	1	1	..	3	2	..	..	1	..	..	..	..	..	78	14.1
	219	226	12	10	4	4	14	19	5	8	5	4	2	1	1	1	535	17.8
	445		22		8		33		13		9		2	1	2			

From table 6 it may be seen that these five females all gave very few offspring, the average being 107, as contrasted with the average of 1,269 given by the normal sisters. This reduction in productivity to one-twelfth that of the normals showed no association with any of the characters involved in the experiment, for the complementary classes were very nearly equal in every case. The termination of life for these five females came sooner; all were dead by the 18th day (table 7) as contrasted with the 29th day for the sisters.

TABLE 7—*Data of table 6 resummarized according to age of mother*

Age period	0	1	2	3	4	5	1,3	1,5	3,4	Total	Total % Recomb.
0– 2........	46	2	..	1	1	1	..	..	..	51	9.8
2– 4........	162	7	3	9	5	4	..	..	..	190	14.6
4– 6........	78	2	2	9	2	1	..	..	1	95	19.0
6– 8........	17	1	1	2	..	..	..	..	1	22	27.3
8–10........	44	3	2	7	1	1	1	..	..	59	27.1
10–12........	29	3	..	2	3	1	..	1	..	39	28.2
12–14........	22	1	..	2	..	..	..	..	..	25	12.0
14–16........	23	2	..	1	..	1	1	..	..	28	21.4
16–18........	24	1	..	..	1	..	..	..	..	26	7.7
Totals........	445	22	8	33	13	9	2	1	2	535	17.8
Per cent Recomb....		4.7	1.5	6.9	2.8	1.9	....				17.8
Standard Recomb....		33.3	1.4	4.3	8.7	12.5	(from table 4)				60.2
Per cent of Standard.		14	107	160	32	15	....				30

The amount of crossing over was likewise greatly reduced; the total of the recombinations (table 7) was only 17.8, as contrasted with 60.2 for the sisters. This reduction to less than a third (30 per cent) of the recombination was characteristic of all five cultures (table 6, last column).

Of the crossovers that did occur, there were two marked departures in type of distribution from the condition shown by the normal sisters. In the first place there was a great reduction of the crossing over in the distal regions but, contrariwise, a slight increase for the mid-region. In the distal sections 1 and 5 the crossing over was only about one-seventh the standard amount shown by the sisters, while in the mid-section, 3, the crossing over was about 60 per cent higher than normal. The intermediate sections, 2 and 4, gave intermediate values of 107 and 32 per cent of the normal values. In the second place, the shape of the curve of recombination according to the age of the mother (table 7, last column) was the reciprocal of that given by the sisters, in that it started low and increased to a maximum at the time the sisters showed a minimum, and thereafter a fall ensued, instead of the sharp rise shown by the sisters.

These differences were clear cut; and the fact that of twelve sisters approximately half showed the aberrant result pointed to the aberration as genetic, with one parent heterozygous.

On the assumption that there was a dominant reducer of crossing over and of productivity, several females from these five cultures were outcrossed to IIIple males (ru h st p^p ss e^s). One female that showed the characters ru st and ss and was heterozygous for h p^p and e^s

$$\left(\frac{\text{ru} + \text{st} + \text{ss} +}{\text{ru h st } p^p \text{ ss } e^s}\right)$$

gave, disregarding the characters ru st and ss that were present throughout: 0, h p^p ss = 66, + = 71; 1, h = 16, p^p e^s = 14; 2, h p^p = 14, e^s = 28; 1, 2, h e^s = 6, p^p = 5. The recombination for h p^p was 18.8, and for p^p e^s

24.3, which were not lower than the 14.9 and 20.7 shown by the normal flies of table 4. Another female, that showed the characters st p^p and e^s and was heterozygous for ru h and ss, gave: 0, ru h ss = 59, + = 86; 1, ru = 18, h ss = 25; 2, ru h = 22, ss = 21; 1, 2, h = 5. The recombinations for ru h and for h ss were 20.3 and 20.3, similar to the 23.8 and 21.3 shown by the normals of table 4. Six original combination females, showing the characters ru st p^p e^s and heterozygous for h and ss, gave the results of table 8. The expected normal value for h ss was 21.3, and only one of the six gave results markedly lower than this. This aberrant culture (12523) gave 15.9, while about 14 was expected for the reduced value, corresponding to the results of table 7. Two further cultures from 12523 gave 7.9 and 13.2 as h ss recombination, and also gave fairly low productivity. Several D ss and other females gave no offspring.

TABLE 8—F_1 *represents offspring of ru st pp* e^s ♀ ♀ *from table 6 and IIIple* ♂ ♂. F_2 *represents similar mating from 12523*

	No.	h ss	+	h	ss	Per cent Recomb.
F_1	12460	118	114	31	26	19.7
	12461	93	100	41	58	33.9
	12462	111	163	41	47	24.3
	12463	150	188	51	74	27.0
	12479	77	98	27	23	22.2
	12523	68	59	9	15	15.9
F_2	12579	10	25	..	3	7.9
	12585	45	54	10	5	13.2

The above results led to the tentative conclusion that the aberrant crossing over was due to a dominant mutant condition, but that this was probably not in the third chromosome; since, of the original combination mothers of the cultures of table 8, one apparently carried the dominant while five did not.

On the assumption that the locus of the crossing-over reducer was in the second chromosome, three females from 12523 were outcrossed to black purple curved males, and wild-type daughters from each F_1 culture were again outcrossed to b pr c males (table 9). The cultures of table 9 were of

TABLE 9—P_1, ♀ ♀ *ex 12523 × black purple curved* ♂ ♂ *(B.C.D.); B.C.,* F_1 + ♀ *ex B.C. or D × b pr c* ♂ ♂

From	No.	b pr c	+	b pr c		b pr	c	b c	pr
B.	12610	127	148	..	..	37	23	..	..
C.	12611	165	180	2	..	40	45	..	2
	12612	124	179	..	..	26	26	1	..
D.	12613	121	169	19	14	30	35	1	2
	12614	146	179	11	16	52	40	3	3
	12615	123	193	11	15	55	40	1	2
	12616	168	203	1	..	37	57	..	..

two types: three represented the normal crossing-over relations, while the other four showed very greatly reduced crossing over for b pr. A pair of wild-type flies from 12610 gave 182 +, 44 b pr c, 12 b pr, 20 c, showing that a dominant suppressor for crossing over between b and pr was present. Another wild-type female was outcrossed to Star Streak black, and the F_1 S Sk not-b flies were bred together (table 10). The absence of recombination classes showed that no crossing over was occurring anywhere in the left limb from Star to black. That the crossing-over reducer was located in the second chromosome and in the left limb of the second chromosome was shown by the fact that S Sk flies from table 10 were inbred for many generations without losing the suppressor of crossing over. The wild-type flies of table 10 and of the subsequent generations were thus homozygous for the C II L, and there was no lethal associated with C II L.

TABLE 10—P_1, + ♀ *ex 12610* × *Star Streak black* ♂ ♂; F_1, *S Sk* ♀ × *S Sk* ♂

No.	S Sk	+
12942	227	111
12943	168	81
12944	163	82

This C II L was then called C II III on the supposition that it was the cause of the aberrant behavior of crossing over in the five females of experiment 1. However, this is certainly not the case; for the low productivity characteristic of those cultures is not present in the stock of S Sk b/C II III. Furthermore, the following test shows that the C II L of this stock does not have the characteristic suppressing action upon crossing over in the third chromosome. A C II III/S Sk b male was outcrossed to a IIIple female and two wild-type F_1 females were backcrossed to IIIple males. The two cultures (16975 and 16976) produced abundantly (623 flies) with recombination classes as follows: 0, 280; 1, 127; 2, 71; 3, 17; 4, 14; 5, 45; 1,2, 9; 1,3, 2; 1,4, 4; 1,5, 27; 2,3, 2; 2,4, 10; 2,5, 12; 3,5, 1; 1,2,5, 1; 1,3,5, 1. The total recombination per section was: 1, 27.4; 2, 18.5; 3, 3.7; 4, 4.5; 5, 14.0. Since standard values for these sections are 26.0, 16.4, 4.2, 10.2 and 12.2, the C II L values are not great departures, except that the p^p ss value of 4.5 is considerably lower than the standard value of 10.2.

The effect of the C II III chromosome on crossing over throughout the second chromosome was tested by backcrossing with dumpy black purple curved plexus speck. The two cultures (16980 and 16981) gave a total of 730 flies, with recombination classes as follows: 0, 421; 1, 0; 2, 0; 3, 111; 4, 165; 5, 33; 3,4, 17; 3,5, 2; 4,5, 1. That is, no crossing over has occurred in the left limb, while crossing over in the right limb was approximately standard in amount.

After the above S Sk b/C II III stock had been run for several generations, with the offspring in the ratio of 2 S Sk : 1 wild-type (which was really homozygous C II III), the nature of the offspring changed in that only S Sk progeny appeared. The crossing-over suppressor was still present but was unable to become homozygous since by mutation a lethal had appeared in the left limb.

The name C II III will perhaps be retained to distinguish the origin of this particular C II L, but the modifier responsible for the aberrant cultures in age experiment 1 has been lost.

Review of "An Effect of Temperature and Age on Crossing Over in the First Chromosome of *Drosophila Melanogaster,*" Stern 1926

The suggestion had been made by Bridges that the special susceptibility of the mid-regions of the autosomes to variations in crossing over was causally connected with the presence of the spindle-fiber attachment in those regions. His work, and that of Plough, on the X-chromosome had failed to show susceptibility to age and temperature. This work was done at a time when it was believed that the spindle-fiber attachment of the X was at the left or yellow end. It was later shown (Anderson, 1925; L. V. Morgan, 1925; Bridges and Anderson, 1925; and Sturtevant (unpublished)) that the attachment of the spindle-fiber is really at the right end of the X. Meanwhile, the localization of the sex-limited mutant bobbed at 70 by Sturtevant furnished a means of testing crossing over relations in the section adjacent to the spindle-fiber. Stern, accordingly, tested the age-effect upon crossing over between Bar and bobbed. He found a slight but significant lowering of the crossing over, that reached its minimum on the fourth day, and was followed by a recovery to normal. The first minimum was slighter in amount of change and earlier in appearance than had been found for chromosomes II and III. Stern also raised females heterozygous for Bar and bobbed (sisters to the flies tested for age change) at a high temperature (30°C). Upon emergence, these heterozygous females were crossed to bobbed males and kept thereafter at normal temperature (25°C). The heat-treated flies showed a marked increase (56 per cent) over the recombination given by the untreated sisters. This effect persisted for about nine days, and then the recombination dropped to normal.

Muller (1925) found that X-rays probably (data meager) caused a decrease in crossing over for the Bar to Beadex section in the right end of the X.

These results strengthen the evidence that the special susceptibility is somehow connected with the locus of the spindle-fiber.

Review of "The Effect of Prolongation of Each Stage of the Life-Cycle on Crossing-Over in the Second and Third Chromosomes of *Drosophila Melanogaster,*" Bergner 1928.

The black to purple section near the middle of the second chromosome and the Dichæte to curled section near the middle of the third chromosome were known from the studies of Bridges and Plough to be especially liable to show change in crossing over with change in the age of the female. They were accordingly utilized in studying the effect upon crossing over of the prolongations of particular stages of the life cycle. F_1 females heterozygous for b pr and for D cu $\left(\frac{b\ \ pr}{+\ +}\ \frac{+\ cu}{D\ +}\right)$ were back-crossed singly to b pr cu males. These pairs were left for three days in a set of culture vials and were then transferred to a fresh set of culture vials at two-day intervals for a total of seventeen days. The percentage of crossing over was calculated for b pr and for D cu for each set of cultures, curves being drawn showing the relation between successive age-periods of the parents and the amount of crossing over shown at successive ages. These data constituted the control data and showed the standard relation, namely, a high initial value, a decline to a minimum and a recovery period.

Experimental data were derived from similar F_1 females that upon emergence were kept from laying eggs for several days by treatment with alcohol or formalin fumes, or by a low diet of sterilized carrot, potato, Pasteur's solution or other media. At the end of the period of temporary enforced sterility the females were mated and were then transferred during seventeen days as the controls had been. In other experiments the pupal period of the F_1 females was prolonged by low temperature or by sealing the culture bottles against escape of CO_2 and entrance of O_2. Similarly the larval period of the F_1 females was prolonged by low temperature, by sealing th culture vials, or by low diets such as banana-agar that had already raised one set of flies, or sterilized carrot, sterilized yeast-agar, etc.

The first conclusion from a comparison of the control and experimental data is that prolongation did produce an alteration in crossing over, whether applied in the adult, pupal, or even in the larval period. This conclusion has the corrolary that in routine experimental work not simply the apparent age after emergence of the flies used for breeding must be standardized, but that these flies should have developed at a standardized rate. Practically speaking, this means that the cultures producing F_1 flies should be maintained at a constant temperature (25°C) and should be well provided with food. The flies that emerge during the first two days are best, and all small flies should be avoided, for small size of adult usually indicates insufficient nourishment during the larval stage, with consequent prolongation of that stage.

The nature of the alteration in crossing over consequent upon lengthening any stage of the period before egg-laying was the premature occurrence of the first minimum, the short duration of this first low, and the early onset of the second high. These alterations were such as to indicate that the changes that normally occur in the ovary of an aging female had not been suspended to the same extent as the changes that determine the age of the soma. Emergence of the adults from the pupal stage may be taken to indicate a definite stage in the development or aging of the soma. But when the age of the germ cells of these just-emerged females was tested by the amount of crossing over it was found that in the case of prolongation of the stages before emergence the crossing-over data corresponded to those of a female considerably older. The correspondence was not strict, for the cycles were shortened and not simply advanced in time.

There was a limit to the independent advance in age of the germ cells, for little if any more alteration was shown by females whose larval period had been prolonged 15 days as compared with those whose larval period has been prolonged only eight days.

Delay in appearance of the alteration when emerged flies were kept from laying for five or more days suggested that eggs in the oöcyte stage had passed the stage at which they were susceptible. Similarly, the time of approximate disappearance of the effect when the larval stage had been prolonged suggests that the early oögonial stage was not affected or that there was subsequent recovery during the later stages. The middle and late oögonial stages seemed to be the period during which the changes in crossing over were effected.

LITERATURE CITED

ANDERSON, E. G., 1925. Crossing over in a case of attached X-chromosomes in *Drosophila melanogaster*. Genetics, **10**:403–417.

BERGNER, A. D., 1928. The effect of prolongation of the life cycle on crossing over in the second and third chromosomes of *Drosophila melanogaster*. Jour. Exp. Zool., **50**:107–163.

BRIDGES, C. B., 1915. A linkage variation in *Drosophila*. Jour. Exp. Zool., **19**:1–21.

———, 1921. Gametic and observed ratios in *Drosophila*. Am. Nat., **55**:52–61.

———, 1927. The relation of the age of the female to crossing over in the third chromosome of *Drosophila melanogaster*. Jour. Gen. Physiol., **8**:689–700.

———, and E. G. ANDERSON, 1925. Crossing over in the X-chromosomes of triploid females of *Drosophila melanogaster*. Genetics, **10**:418–441.

———, and T. H. MORGAN, 1923. The third-chromosome group of mutant characters of *Drosophila melanogaster*. Carnegie Inst. Wash. Pub., **278**:123–304.

CLAUSEN, R. J., 1924. The inheritance of cinnabar eye-color in *Drosophila melanogaster*, including data on the locus of jaunty. Jour. Exp. Zool., **38**:423–436.

MORGAN, L. V., 1925. Polyploidy in *Drosophila melanogaster* with two attached X-chromosomes. Genetics, **10**:148–178.

MULLER, H, J., 1916. The mechanism of crossing over. Am. Nat., **50**:193–221, 284–305, 350–366, 421–434.

———, 1925. The regionally differential effect of X-rays on crossing over in autosomes of *Drosophila*. Genetics, **10**:470–507.

———, 1926. Induced crossing-over variation in the X-chromosome of *Drosophila*. Am. Nat., **60**:192–195.

PLOUGH, H. H., 1917. The effect of temperature on crossing over in *Drosophila*. Jour. Exp. Zool., **24**:147–209.

———, 1921. Further studies on the effect of temperature on crossing over. Jour. Exp. Zool., **32**:187–202.

STERN, C., 1926. An effect of temperature and age on crossing over in the first chromosome of *Drosophila melanogaster*. Proc. Natl. Acad. Sci., **12**:530–532.

STURTEVANT, A. H., 1913. The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. Jour. Exp. Zool., **14**:43–59.

WEINSTEIN, A., 1918. Coincidence of crossing over in *Drosophila melanogaster* (ampelophila). Genetics, **3**:135–173.

III

DEFICIENT REGIONS OF NOTCHES IN DROSOPHILA MELANOGASTER

By Ju Chi Li and Calvin B. Bridges

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INTRODUCTION

One of the most frequently recurrant mutants is the Notch character in *Drosophila*. Twenty-five Notches have been found, four of which were still kept in stock in Columbia University Laboratory up to the time when the following experiment was started in September, 1923. A linkage test of these four Notches (N8, N11, N18, N19) leads to the conclusion that the lengths of the deficient regions in these Notches are different; and the differences are somewhat correlated with the general characteristics of the mutants.

THE EXPERIMENT

The first "Notch" mutation was found in 1914 by Dexter (1914) who showed that the character was sex-linked, dominant in the female, and lethal in the male. Bridges soon afterward found a second "Notch," and located at 1.5 units to the right of the white locus (Morgan and Bridges, 1916). Several other Notches were found by Morgan, Bridges, Muller and Gowen. One, Notch-6, found by Bridges, showed a new characteristic for Notch, *viz.*, "pseudo-dominance" for facet (Metz and Bridges, 1917). When the Notch-6 female was crossed to facet males, all of the Notch-6 daughters were at the same time facet. This similarity to the behavior of the forked in f-B deficiency (Bridges, 1917) and vermilion in v-deficiency (Bridges, 1919) at once suggested that Notch was a deficiency that included the locus for the facet character. It was seen that both the facet character and the Notch character in these hybrids were "exaggerated," *i. e.*, showed a more extreme departure from the wild-type, while the infertility was exaggerated to such an extent that not one of the eighteen Notch-facet daughters produced offspring.

Notch-8, found by Mohr in 1918, has been the object of very extensive study (Mohr 1919, Mohr 1923). Notch-8 showed all the phenomena encountered in the previous Notches, but differed from them in involving a longer section. Thus, Notch-8 gave pseudo-dominance and exaggeration with facet, with white and its eleven allelomorphs located 1.2 to the left of facet, and with Abnormal located 1.8 units to the right of facet. This interval of approximately three units is a minimum measure of the length of the deficient section. Very extensive linkage tests showed that crossing over was entirely eliminated from the deficient section, and that the map of the deficient X is 3.8 units shorter than the standard map.

Of the twenty-five Notches, four (N8, N9, N18, N21) have been shown to be extensive enough to cover the locus of white. Two of these, the Notch-8 of Mohr and Notch-18, found by Bridges, are used for the present studies. Two other Notches, Notch-11 and Notch-19, both found by Bridges, are used as examples of Notches of shorter extent of the deficient section. A short account of Notch-11 has been included in the paper of Mohr (Mohr, 1923). Notch-18 was shown by Bridges (unpublished data) to cover the locus of white, and to give indications, through linkage, of being a still more extensive deficiency than Notch-8. Notch-18 was tested and found to have little if any effect upon crossing over in other sections of the X. Notch-19 seemed to be an especially short deficiency, and was characterized by excellent viability.

The previous work had thus indicated that the deficient sections of the four Notches are not equal in length. In order to determine more accurately the difference in length of these deficient sections, the following conditions must be observed:

1. The four Notches must be crossed to males from the same stock, that carries mutant factors both to the right and to the left of the Notch section. Since there is no crossing over in a deficient section (Bridges 1917, Mohr 1919, 1923), the length of such a section should correspond to the number of units (or percentage of crossing over) by which the map for the deficient X is shorter than the map for not-Notch flies.

2. Notch and control cultures must be treated alike, with the same temperature and moisture conditions, and must be raised on the same kind of food, as any environmental difference may possibly influence the percentage of crossing over.

3. Large numbers of progeny must be raised for each generation, and the experiment must be continued by breeding to the same inbred stock for a number of generations, so as to make the modifiers of the four Notches, and also of the amount of crossing over, as nearly alike as possible.

To fulfill the above conditions, a stock was made up consisting of four mutant characters, *viz.*, scute (0.±), broad (0.6), echinus (5.5), and ruby (7.5). Scute and broad are known to lie to the left of all the deficient sections, and echinus and ruby to the right.

The males of the quadruple-recessive stock were then mated to females of the four Notch stocks. From seven to twelve cultures for each Notch were started at approximately the same time, and raised in the same incubator, wherein a temperature of 24–25 degrees centigrade has been maintained constantly. The progenies of these crosses were then classified and recorded. From these P_1 cultures, one culture for each of the four series of the Notches was selected as the stock from which Notch females were again taken to cross to the quadruple-recessive males in the same fashion as at first.

The results of such crosses in six successive generations are recorded in tables 1 to 4. In these tables "0" represents the total of non-crossover flies; 1 represents the total of flies due to crossing over in the first section, namely sc-br; 2, in the second section. br-N; 3, in the N-ec section, and 4, in the ec-rb section.

In the meantime, a quintuple-recessive stock was made up with the mutant character "apricot" added to the original quadruple-recessive stock. Tables 5 to 8 give the results of two successive crosses of the four different Notches with the quintuple-recessive males. As both Notch-8 and Notch-18

TABLE 1—F_1 to F_6, *Notch-8* ♀ × *sc br ec rb* ♂

Generation	0	1	2	3	4	Totals
1	1,206	0	6	10	8	1,230
2	1,258	0	2	1	14	1,275
3	1,958	1	5	4	20	1,988
4	775	0	3	0	7	785
5	1,052	3	3	2	18	1,078
6	1,637	0	3	1	16	1,657
Totals	7,886	4	22	18	83	8,013
Percentages	....	0.05	0.27	0.22	1.04	1.58

TABLE 2—F_1 to F_6, *Notch-18* ♀ × *sc br ec rb* ♂

Generation	0	1	2	3	4	Totals
1	888	0	0	2	5	895
2	1,303	0	3	6	6	1,318
3	1,755	0	0	3	18	1,776
4	761	0	0	1	1	763
5	1,197	0	0	1	11	1,209
6	1,163	0	0	0	2	1,165
Totals	7,067	0	3	13	43	7,126
Percentages	....	0	0.04	0.18	0.60	0.83

TABLE 3—F_1 to F_6, *Notch-11* ♀ × *sc br ec rb* ♂

Generation	0	1	2	3	4	Totals
1	1,211	0	50	31	22	1,314
2	1,495	0	42	38	25	1,600
3	1,697	1	28	24	21	1,771
4	573	1	13	6	7	600
5	1,011	0	13	23	15	1,062
6	1,046	2	15	17	9	1,089
Totals	7,033	4	161	139	99	7,436
Percentages	....	0.05	2.17	1.87	1.33	5.42

TABLE 4—F_1 to F_6, *Notch-19* ♀ × *sc br ec rb* ♂

Generation	0	1	2	3	4	Totals
1	1,069	0	31	25	20	1,145
2	1,349	0	14	26	21	1,410
3	1,698	0	36	27	23	1,784
4	384	0	6	5	6	401
5	1,076	0	21	25	15	1,137
6	1,184	0	11	10	18	1,223
Totals	6,760	0	119	118	103	7,100
Percentages	....	0	1.68	1.67	1.45	4.80

cover the locus for white, and "apricot" is one of the eleven allelomorphs of white, all the Notch females from the crosses involving the above two Notches showed apricot eye-color and produce four kinds of crossover classes. But in cases where the Notch-11 and Notch-19 were involved, the Notch females were red eyed; and consequently we can distinguish five different crossing-over sections in such crosses.

TABLE 5—*F_7 and F_8, Notch-8* ♀ × *sc br w^a ec rb* ♂

Generation	0	1	2	3	4	Totals
7	1,917	0	3	6	19	1,945
8	1,863	0	0	3	21	1,887
Totals	3,780	0	3	9	40	3,832
Percentages	....	0	0.08	0.24	1.04	1.36

TABLE 6—*F_7 and F_8, Notch-18* ♀ × *sc br w^a ec rb* ♂

Generation	0	1	2	3	4	Totals
7	1,899	0	1	2	11	1,913
8	879	0	0	2	10	891
Totals	2,778	0	1	4	21	2,804
Percentages	....	0	0.04	0.14	0.75	0.93

TABLE 7—*F_7 and F_8, Notch-11* ♀ × *sc br w^a ec rb* ♂

Generation	0	1	2	3	4	5	Totals
7	1,733	1	14	23	42	28	1,841
8	1,483	1	5	14	40	23	1,566
Totals	3,216	2	19	37	82	51	3,407
Percentages	....	0.06	0.56	1.09	2.41	1.49	5.61

TABLE 8—*F_7 and F_8, Notch-19* ♀ × *sc br w^a ec rb* ♂

Generation	0	1	2	3	4	5	Totals
7	1,855	0	14	28	55	46	1,998
8	1,200	2	4	14	47	20	1,287
Totals	3,055	2	18	42	102	66	3,285
Percentages	....	0.06	0.55	1.28	3.10	1.89	6.97

In this connection, it is interesting to note that side by side with the low production in the number of progeny in the cultures of Notch-18, there were manifestations of embryonic disturbances which resulted in two types of malformations. At least six Notch flies that had "split thorax" and about thirty Notch flies that showed "defective eyes" were found during the course of the experiment. The character "split thorax" was fairly definite, and constant. The flies invariably had two deformed half thoraxes united by their borders, the juncture being devoid of bristles and hair. The "defective eye," on the other hand, was a very variable character. It sometimes affected one eye and sometimes both; and the effect was sometimes slight, resulting in one eye which was smaller than the other; sometimes a large part of the eye was lost and a tuft of black hair had taken its place. As stated before, these malformations were found only in the Notch females; and only in the cultures of Notch-18 did they seem to be prevalent, though a few of the "defective eye" malformations cropped out in cultures of Notch-8 and Notch-19. The flies were tested by different crosses; but the "split-thorax" flies did not breed. Those with "defective eye" did, but the reappearance of this character in the progeny was infrequent and again restricted to the Notch females. It is highly probable, therefore, that these malformations were nothing but embryonic phenomena, induced by the abnormal chromosome in the Notch flies; that is, they were variable effects of the more extreme Notch mutations.

That this chromosome favors abnormal occurrences was indicated by the fact that quite a number of intersexes were found in these cultures. These were similar to those reported by Bridges (Bridges, 1921) and shown to be due to two X-chromosomes balanced against three sets of autosomes. The first intersex was found in a Notch-8 culture, December 1, 1923. It carried the scute and broad characters and Notch wings. Its abdomen and genitalia were male-like. It was first noticed from the fact that it appeared to be a male with typical Notch wings, the Notch being ordinarily restricted to the female and lethal in the male. After diagnosis as an intersex, it was mated to a yellow female from the attached-X stock and was proved to be sterile by consecutive matings to three such females. Another intersex was found from a culture of Notch-11, January 2, 1924. It also bore Notch wings. A third intersex similar to the second was recorded January 12, 1924. It came from a culture of Notch-8. The fourth intersex was recorded February 14, 1924. It came from a cross between Notch-18, which carried four recessives, and a quintuple recessive male. This fly showed male abdomen and sex-comb, but Notch wings and very light apricot eyes.

The following tables give the summaries of the linkage results:

TABLE 9—F_1 *to* F_6, *Notches-8, -18, -11, -19* ♀ × *sc br ec rb* ♂

	0	1	2	3	4	Total	Per cent				
							1	2	3	4	Total
N- 8..........	7,886	4	22	18	84	8,013	0.05	0.27	0.22	1.04	1.58
N-18..........	7,067	0	3	13	43	7,126	0	0.04	0.18	0.60	0.83
N-11..........	7,033	4	161	139	99	7,436	0.05	2.16	1.87	1.33	5.42
N-19..........	6,760	0	119	118	103	7,100	0	1.68	1.67	1.45	4.80

TABLE 10—*F_7 and F_8, Notches-8, -18, -11, -19 ♀ × sc br w^a ec rb ♂*

	0	1	2	3	4	5	Total	Per cent					
								1	2	3	4	5	Total
N- 8..	3,780	0	3	....	9	40	3,832	0	0.08	......	0.24	1.04	1.36
N-18..	2,778	0	1	....	4	21	2,804	0	0.04	......	0.14	0.75	0.93
N-11..	3,216	2	19	37	82	51	3,407	0.06	0.56	1.09	2.41	1.49	5.61
N-19..	3,055	2	18	42	102	66	3,285	0.06	0.55	1.28	3.10	1.98	6.97

TABLE 11—*Summary of tables 9 and 10*

	0	1	2	3	4	Total	Per cent				
							1	2	3	4	Total
N- 8......	11,666	4	25	27	123	11,845	0.03	0.21	0.23	1.04	1.51
N-18......	9,845	0	4	19	64	9,930	0.00	0.04	0.17	0.65	0.86
N-11......	10,249	6	217	221	150	10,843	0.06	2.00	2.04	1.38	5.48
N-19......	9,815	2	179	220	169	10,385	0.02	1.72	2.12	1.63	5.49
N- 8 (Mohr, 1923).		...	.....	.....	.....	15,000+	0.6	0.9	0.2	1.9	3.60

DISCUSSION AND CONCLUSIONS

As seen in table 11, there are clear differences between the results here obtained and those obtained earlier by Mohr (Mohr, 1923). The sc-br value is here 0.03 as compared with 0.6, the br-N8 value is 0.21 as compared with 0.9, and the ec-rb value is 1.04 as compared with 1.9. The values obtained by Mohr agree with standard expectation, while the values here shown are significantly lower. For the whole interval from sc to rb, there was a difference of 2.1 units. Since the different experiments reported here agree with one another very closely in the sc-br sections, it is probable that the stock common to them all, *viz.*, sc br ec rb, is responsible for the difference between these and the earlier results of Mohr. The sc br ec rb stock was derived from the sc br ec rb t f stock used by Mohr in his crosses with N-8. But the region to the right of the ruby was replaced, through crossing over, by a corresponding piece from an X of a wild stock. This piece evidently contained a dominant modifier of crossing over. The sc br w^a ec rb stock contains this same modifier, and was in fact derived from the sc br ec rb stock. Bridges encountered this modifier in using the sc br ec rb stock in other linkage experiments (unpublished results), and since the same stock was used here, it is certain that the above interpretation of the secondary reduction is substantially correct. The modifier was not discovered however before the present experiments were under way.

The presence of the modifier makes a comparison of the results with the standard amounts of crossing over less valuable. But since the modifier was present throughout, the different experiments can be compared with one another safely. It is seen that Notch-18 makes the greatest reduction from the standard, viz., 0.6 unit more than Notch-8 does. Also the great inviability, lower productivity, more frequent sterility and more marked development of the notching and other characteristics all agree with the idea that the extent of the deficient section is greater in Notch-18 than in Notch-8. The reduction of Notch-11 and of Notch-19 are equal, *viz.*, 2.0, practically all of which may be attributed to the action of the modifier, since with Notch-8 the modifier made a difference of 2.1 units between the normal results of Mohr and the modified results. However, the greater viability, and productivity and less frequent sterility of Notch-19 may be interpreted to mean that the extent of the Notch-19 deficiency is less than that of Notch-11, and of course still less than the deficiencies of Notch-8 and Notch-18.

BIBLIOGRAPHY

BRIDGES, C. B. 1917. Deficiency. Genetics 2:445–465.
———. 1919. Vermilion Deficiency. Jour. Gen. Physiol. 1:645–656.
———. 1921. Triploid Intersexes in *Drosophila melanogaster.* Science, n.s., 54:252–254.
DEXTER, J. S. 1914. The Analysis of a Case of continuous Variation in *Drosophila* by a Study of its Linkage Relations. Am. Nat. 48:712–758.
METZ and BRIDGES. 1917. Incompatibility of Mutant Races in *Drosophila.* Proc. Nat. Acad. Sci. 3:673–678.
MOHR, O. L. 1919. Character Changes caused by Mutation of an entire Region of Chromosome in *Drosophila.* Genetics 4:275–282.
———. 1923. A genetic and cytological Analysis of a Section Deficiency involving four Units of the X-chromosome in *Drosophila melanogaster.* Zeits. ind. Abst. Vererb. 32:108–232.
MORGAN and BRIDGES. 1916. Sex-linked Inheritance in *Drosophila.* Carnegie Inst. Wash. Pub. No. 237.

IV

EXCEPTIONAL SEX-RATIOS IN CERTAIN MUTANT STOCKS WITH ATTACHED X'S

By T. H. Morgan

EXCEPTIONAL SEX-RATIOS IN CERTAIN MUTANT STOCKS WITH ATTACHED X'S

By T. H. Morgan

The most frequent change in the normal sex-ratio of *Drosophila melanogaster* is due to the appearance of a sex-linked, recessive lethal gene in the X-chromosome of a female. Half the sons of females heterozygous for this gene die. The result is a ratio of two daughters to one son. The presence of two different lethal genes in the X-chromosomes of a female, one in each X, may give only daughters and no sons, or few, or several, sons. Only daughters result when the lethal genes in opposite chromosomes are so nearly at the same level that crossing over does not occur, hence an egg having one or the other X, and fertilized by a Y-sperm, does not come through. A few sons result when crossing over occurs between the two lethals leaving one X-chromosome with neither lethal gene. Any such egg, retaining this X, fertilized by a Y-sperm, produces a viable male. The number of these males will depend on the "distance" apart of the two lethal genes in question, or in other words, on the amount of crossing over that takes place. In addition to these cases with completely lethal genes there are a few other cases (*see* Bridges, 1919) where a sex-linked recessive gene affects the male to a less extent; hence, while lowering the relative number of males, allows a few lethal males to come through.

It is the reverse situation that is to be described here, where more sons than daughters are produced. This gives the impression of a high male ratio, while in reality there is a deficiency of females. The actual number of sons is not greater than in ordinary pair cultures but there are fewer daughters. Sporadic cases of high male ratios (or more accurately low female ratios) have appeared from time to time in our cultures, but have not, heretofore, been studied to determine the cause of the exceptional results.[1] Four years ago, while working with stock having in the female line two attached X-chromosomes, each carrying at that time a gene for yellow wings (and body color), certain cultures were met with that gave many males and a few females. It is a peculiarity of this stock, having attached X's (and also a Y-chromosome), that the daughters receive their two attached X's from their mother and are, therefore, in the present case, yellow; and the sons get their single X from their father (the "female-producing" sperm) and the Y from their mother; hence the males are fertile and show the characters whose genes are carried by the X-chromosome of the father. The males, then, are strictly patroclinous with respect to the sex-linked characters of the father, but have a maternal as well as a paternal set of autosomes.

It happened at the time when these exceptional ratios appeared that the males used had Bar eyes. These bred to yellow females with attached X's gave round-eyed, yellow daughters (with attached X's) and Bar-eyed, gray sons. The number of daughters and sons was very variable. At one extreme there were no daughters at all, and at the other extreme the daughters were almost as numerous as the sons. This great variability in the sex-ratios has made the analysis of the case extremely difficult. Some of the ratios that have appeared are given in table 1.

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[1] Mohr, O. L., and Sturtevant, A. H., Proc. Soc. Exp. Biol. Med., XVI, 1919; Thompson, D. H., Anat. Rec. XX, 1920; Bonnier, Acta Zool. III, 1922; Bonnier, G., Zeit. ind. Abst.-Vererb. XXX, 1923; Redfield, H., Genetics XI, 1926; Li, J. C., Genetics XII, 1927. The hybrid male ratios of *D. simulans* and *D. melanogaster* involve a different situation.

TABLE 1—*Offspring of yellow females with attached X-chromosomes by various Bar males*

yy ♀	Bar ♂	yy ♀	Bar ♂	yy ♀	Bar ♂	yy ♀	Bar ♂
(1) 0	55	2	30	2	4	2	22
(1) 0	15	3	39	2	107	2	26
(1) 0	13	(1) 3	65	4	12	2	70
(1) 5	24	5	70	6	20	3	40
5	23	5	125	7	51	4	87
6	143	7	48	7	91	4	130
11	84	8	165	7	233	7	17
11	22	10	53	8	106	10	64
11	104	12	81	10	39	13	149
12	100	13	108	14	76	14	152
(1) 13	46	14	186	14	158	15	79
15	44	15	138	(1) 17	128	16	36
15	117	17	72	23	203	16	96
15	138	18	44	33	125	24	132
20	100	(1) 18	55	33	230	30	94
21	108	19	80	36	88	68	94
30	169	19	106	38	173	(1) 3	33
30	112	20	109	49	241	(1) 6	17
31	187	20	147	55	78	7	141
(1) 34	140	20	84[a]	90	26	(1) 15	66
35	64	23	92[a,b]	5	6	27	115
(1) 36	319	24	118	7	15	55	104
38	110	24	151[c]	21	84	3	53
41	123[a]	24	24[b]	5	74	3	76
47	179	26	127	0	77	3	112
52	133	26	181	5	10	27	140
53	178	30	113	(1) 12	88	40	94
..	...	33	235	28	203[c]	41	98
..	...	34	81	39	62[b]	48	69
..	...	35	98	(1) 92	103	51	240
..	...	36	286	2	70	134	212
..	...	36	212	29	97[c]	9	38
..	...	38	209	1	11	25	160
..	...	47	230	3	47	26	123
..	...	55	141	13	73	26	130
..	...	59	169	23	90	32	58
..	...	84	201	82	187	45	169
..	...	..	...	101	198	66	203

[a] One yellow male in addition from a "broken" double X-chromosome.
[b] One XXX female.
[c] One heterozygous Bar gray ♀.

The data given in table 1 are only a small part of those on record, but an unselected part. They serve to show the very wide variability of the sex-ratios; but they are imperfect in the sense that most of the records are from small mass-cultures in which about 3 to 10 virgin females and as many males were used. The results are, however, not different from those in which a single female (with one or a few males) was isolated. Such cultures are indicated in the table by the superscription (1). Numerous other pair matings not here recorded confirm this conclusion. In one respect, however, results from pair matings are under certain conditions expected to give somewhat different results from those of small mass cultures. If a single female does not promptly lay her eggs the food is not so well worked over by the larvæ as when more eggs are laid and more larvæ are present at the start. Conversely, if too many larvæ are present, competition (a term that does not pretend to explain the nature of the process) may result in the elimination of the weaker types. These possible effects do not appear to seriously influence the general character of the output in the present case as the similarity of pair and mass cultures shows.

The sex ratios are, as stated, highly variable. The lowest ratios are those where no females appear at all, but these are not characteristic since most such cases contain relatively fewer individuals. On the other hand, the ratios when several or many females are present, run approximately 2 : 1 and 3 : 1, and 4 : 1 and 5 : 1, etc. That these ratios approximating to whole numbers are, so to speak, "accidental" rather than characteristic, was shown by keeping records of preceding generations. In nearly every case recorded in table 1 such records are known (but not given here). Inspection of these "lines" shows the same variability as that shown in the table. A few examples are as follows: A culture that gave 3 ♀ to 39 ♂ came from one 25:180; this from 21:75; and this from 23:130. Another that gave 36 ♀ :212 ♂ came from 9:107; 7:28; 13:45. Another that gave 23 ♀ :92 ♂ (plus 1 yel. ♂) came from 24:101; 14:105; 7:76. Another that gave 11 ♀ :104 ♂ came from 0:57; 15:35; 24:201; 16:251. Another that gave 13 ♀ :46 ♂ came from 0:57; 15:34; 15:147; 12:93; 7:233.[1] One of the more extreme records, where there was an apparent return to equality, namely 24 ♀ :24 ♂ (pair culture) came from 20:126; 18:34; 18:39; 17:52; 26:41. A culture was made up from the 24:24 bottle. It gave 4 ♀ :7 ♂ (plus one XXX). The one-to-one ratio may have been only an extreme fluctuation. As indicated in a footnote to this table there were a few instances in which XXX females came through. These are the individuals in which an egg, retaining the attached X's, is fertilized by an X-sperm. It is known that such individuals die, as a rule, but occasionally one or more survives. The contrary class of YY-males never survives. The presence of one X at least is essential for survival.

There are a few records in the table of individuals that have arisen after the breaking apart or separation of the attached X's. Such cultures are marked with an [a]. When one X was left in the egg (with or without the Y-chromosome) and this egg was fertilized by an X-sperm, carrying the bar gene, a female resulted that had normal body color (since one "gray" or wild-type gene dominates yellow), and was heterozygous for Bar eyes. If the same kind of egg is fertilized by a Y-sperm, a yellow male results (since the broken X in the egg carries the yellow gene). Some of these yellow males have been utilized to make up yellow stock, carrying two separate X's, in order to see whether the high sex ratios carry over in such unattached X's (see below).

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[1] The lines in which no females are here recorded were continued from later females that hatched in the same cultures.

OUT-BREEDING ATTACHED-X FEMALES; LOW FEMALE-PRODUCERS

The low female-producing females have been bred at different times to males of other stocks (having normal ratios) in order to find out whether the eggs are entirely responsible for the exceptional ratios. Three virgin females bred, in separate cultures, to Bar males from Bar stock gave respectively 16 : 25, 19 : 69, and 50 : 156. These ratios compare favorably with those in table 1, although none are as high as the highest there. In a fourth case an attached-X yellow female (from a culture giving 7 : 132) was mated to a vermilion-eyed male and gave 25 : 50. In a fifth case the same combination gave 26 : 26. In a sixth case a female (from a culture giving 41 : 101) was bred to an eosin stock male and gave 18 : 69. In two other cases, females were bred to Star Curly Dichæte males of stock and gave respectively 20 : 85 and 2 : 12. In another case a "low" female was bred to an eosin forked male and gave 19 : 79. In five cases females were outbred to males of other stocks (characters not recorded) and gave respectively 29 : 156; 26 : 26; 59 : 174; 13 : 90; 73 : 39. The last case cannot be explained on the assumption that a sex-linked recessive lethal that affects the male had appeared in the mother, because she has two X's and neither passes into the male, nor in the father unless it were present in a great many sperm-cells. The offspring were not tested. Another female was bred to eosin forked stock males and gave 18 ♀ :69 eosin forked ♂. Another female to vermilion male gave 76 : 124 (plus 20 XXX females), and another female to eosin cut vermilion males gave 72 : 90 (plus 12 XXX females). The occurrence of such XXX females has since been shown by Sturtevant to be often connected with a moderately low temperature and with heterosis.

At a later date (1925) the same experiment was repeated with low female-producing yy-stock bred to males of other stocks (vermilion and Star Dichæte) giving normal ratios. One female only was present in each culture. In the next table the results are recorded.

yy ♀ from low ♀ ratio stocks by v ♂

yy ♀	v♂	XXX ♀
13	46	
8	37	
14	17	
7	31	
36	79	4
21	56	1
29	81	1
16	28	
42	85	
23	58	1
209	558	7

The low female ratios are again apparent. In one case, however (not here recorded), in which eosin singed males were used there were equal numbers of yy females (457) and eosin singed males (432). There is a suspicion that the record belongs to a cross where stock (not low female producing) yy-females had been used. This experiment was, therefore, repeated in 1927, and at the same time, for control, other tests were made with four other kinds of males (Lobe, vermilion, cut 6, Star Curly Dichæte). The results are recorded in the following tables.

yy ♀ from low ♀ ratio stocks S Cy D ♂

yy ♀	♂
0	38
46	85
48	84
53	94
38	71
16	44
18	71
25	72
60	84
45	63
41	89
65	98
28	102
16	53
10	49
509	1,097

low yy ♀ by w^esn ♂ (from yy stock)

	yy ♀	w^esn ♂
yy ♀ from *A* (41 ♀ – 116 ♂)....	0	27
	1	11
yy ♀ from *B* (73 ♀ – 143 ♂).....	0	3
	3	97
yy ♀ from *C* (63 ♀ – 128 ♂).....	0	79
	8	41
	10	36
yy ♀ from *D* (67 ♀ – 111 ♂)....	0	40
	3	11

low yy ♀ by 2 Lobe ♂

	yy L ♀	L ♂
yy ♀ from *A* (41 ♀ – 116 ♂)....	16	27
	0	12
yy ♀ from *B* (73 ♀ – 143 ♂)......	17	109
yy ♀ from *C* (63 ♀ – 128 ♂).....	0	24
	10	74
	33	110
yy ♀ from *D* (67 ♀ – 111 ♂)......	67	111

low yy ♀ by 2 v ♂

	yy ♀	v♂
yy ♀ from *B* (73 ♀ – 143♂)......	0	7
yy ♀ from *D* (67 ♀ – 111♂)......	0	14

low yy ♀ by 2 ct^6 ♂

	yy ♀	ct♂
yy ♀ from *A* (41 ♀ – 116♂).....	32	69
	0	7
yy ♀ from *B* (73 ♀ – 143♂).....	9	21
	7	29
yy ♀ from *C* (63 ♀ – 128♂).....	0	14
	3	134

1 low yy ♀ by 2 S/Cy D ♂

	yy ♀				♂			
	S	SD	Cy D	Cy	S	SD	Cy D	Cy
yy ♀ from *A* (41 ♀ – 116♂).....	2	3	2	0	16	13	10	13
	4	0	0	7	42	1	1	34
yy ♀ from *B* (73 ♀ – 143♂)....	1	0	0	0	3	1	8	2
	0	0	0	0	2	0	1	0
yy ♀ from *D* (67 ♀ – 111♂).....	2	0	0	1	28	23	13	17

The deficiency in females is everywhere apparent, including the experiment with eosin singed males.

These ratios leave no doubt that the attached-X females of these stocks, highly inbred for low female ratios, give low female ratios in the first outcross, and many of these ratios appear to be as extreme as those of the inbred stocks, despite the fact that a new half-set of autosomes has been introduced.

SEX-RATIOS OF THE SECOND GENERATION FROM LOW RATIO ATTACHED-X FEMALES

Three kinds of tests were made of the F_1 yellow attached-X females from the preceding cross: (A) Some of them were mated in pairs (or in small numbers) to their F_1 brothers; (B) others were mated (back-cross) to ordinary stock males; (C) others were mated to males of attached-X stock.

A. In table 2, the F_2 data are given. It is obvious that low female ratios prevail, and that they average about the same as those of the attached-X stock. The F_1 females that gave these results had the attached-X of their mothers, but the Y-chromosomes came from the other stock and the autosomes were half-and-half. It follows that the disturbance, if not due to the attached-X's alone, cannot be due to autosomal, recessive intensifier genes in the low female stock. It remains to be shown whether or not a dominant gene (or genes) might be present in the autosomes of the attached-X stock.

TABLE 2

	yy ♀	Bar ♂	yy ♀	Bar ♂
A	18	29	24	42
	31	45	19	36
	4	12	94	347
	33	104	16	39
	36	107	52	140
	18	75	..	..
	8	75	..	..
	8	59	..	..
	4	44	..	..

B. The results after outcrossing twice, table 3, to a foreign male (of normal ratio stock) are less consistent than in the last case. Some of the ratios are as low as before (or even lower), but there are more that approach nearer to equality, and, in addition, there are several cultures in which equality of the sexes occurs. Here again the attached-X's are the same as in the P_1 and F_1; and the F_1 female is heterozygous for the autosomes as in the last cross (A). Since the lowest ratios are the same in both cases, two inferences are possible: (1) that the mothers of the broods of table 3 did not all have the same composition, while those in table 2 were all alike, or (2) that through the recombination of autosomes in the eggs of the F_1 female the results are affected. If this is the explanation, it is difficult to harmonize it with (A), for some of the same recombinations would occur there also (after extrusion of the polar bodies).

TABLE 3

	yy ♀	♂	yy ♀	♂
B	95	97	112	161
	57	101	92	149
	108	122	15	32
	54	71	57	137
	59	102	14	85
	33	47	14	158
	81	73	21	24
	81	75	26	21
	31	24	9	56
	8	25	48	94
	25	117	9	119
	38	49	13	57
C	yy ♀	Bar ♂		
	1	49	..	..

C. Only one test was made in which the F_1 female was mated to a male of low female producing attached-X stock. It gave a very low female ratio (1 ♀ : 49 ♂).

This experiment was repeated later using F_1 females obtained from a cross to the dominant mutant Lobe; also two others from F_1 females from a cross to eosin singed. The F_1 female was back-crossed to a corresponding male from low-female producing yy stock. The results are given in table 3*a*.

TABLE 3*a*

F_1 yy ♀ (from D. low ♀ by Lobe ♂) by low ratio ♂ (from O. (52-101))

F_2	yy ♀	♂
	50	110
	10	78

F_1 yy ♀ (from D. low ♀ by Lobe ♂ (34-119)) by low ratio ♂ (from M. (33-111))

F_2	yy ♀	♂
	30	175

F_1 yy ♀ (from C. low ♀ by Lobe ♂ (11-76)) by low ratio ♂ (from M. (33-111))

F_2	yy ♀	♂
	25	246

F_1 yy ♀ (from C. low ♀ by w^esn ♂ (13-44)) by low ratio ♂ (from M. (33-111))

F_2	yy ♀	♂
	3	23

F_1 yy ♀ (from C. low ♀ by w^esn ♂ (13-44)) by low ratio ♂ (from O. (52-101))

F_2	yy ♀	♂
	54	88

Low female ratios are again present. These back crosses do not show whether or not there are dominant autosomal factors involved, because the low ratio males themselves would introduce into the zygote these factors if they exist.

SEX-RATIOS FROM MATING ATTACHED-X FEMALES OF YY-STOCK (NOT LOW RATIO FEMALES) TO MALES OF LOW RATIO STOCK

We keep several stocks of yellow attached-X flies from which the aberrant low producers were originally derived. In order to test whether the males of the low female line can introduce a change in sex-ratio when bred to the stock attached-X females (not low), the following experiments were made. Unfortunately, as the sequel will show, it was later found that in the stock of the original attached-X's there arise occasionally females that give low female ratios. This condition introduces a disturbing element into the situation.

TABLE 4

yy ♀	Bar ♂	yy ♀	Bar ♂
57	83	83	136
99	103	47	53
56	101	47	66
67	89	65	89
114	100	138	190
61	81	35	21
61	80	66	83
76	115	43	59
56	64	62	68
45	73	80	153

The first generation is given in table 4. In most of the cultures it is found that females run somewhat behind the males. This may be explained in part as due to the lower viability of yellow flies in competition with normals, here the males; but in some of the cultures the males are nearly twice as numerous as the females. The absence of extremely low female ratios is conspicuously apparent in contrast with the preceding cross where the low females were the parents.

Second generations were also obtained by inbreeding brothers and sisters from some of the F_1 offspring. The F_1 pairs were not consciously selected from the low cultures, because at the time of making the matings the final counts had not been made. The results are given in table 5.

Table 5—*F_1 attached-X (yy) ♀ by F_1 Bar ♂ (out of yy stock and high Bar ♂)*

F_2		F_2		F_2	
yy ♀	♂	yy ♀	♂	yy ♀	♂
64	56	54	63	37	41
60	69	29	17	11	10
42	47	39	45	28	27
90	80	68	85	62	110
74	68	58	85	..	..
87	57	21	31	..	..
35	54	69	87	..	..
13	22	44	63	..	..
..	..	48	43	..	..

The F_2 ratios are like those of the F_1's except that there are more of the one-to-one ratios.

Another test was made by back-crossing the F_1 ♀, obtained as above, to males of low female stock (table 6).

Table 6—*F_1 yy ♀ by high ratio Bar ♂ (out of yy stock ♀ by high ratio Bar ♂)*

F_2 yy ♀	22	28	29	49	88	16	31	16	18
F_2 ♂	34	28	45	55	102	46	59	159	276

There are at least three ratios that fall well within the limits of the low female-producing line, and they indicate either that certain autosomal combinations have here been brought in through the males, or else that low producing factors were still present in the yy stock. The latter explanation is probably correct as later tests have shown.

Another consideration may now be brought forward. If a low ratio factor is present in the double X-chromosome, and is recessive, it may be present in some females of the double yellow (not low) stock in only one of the X's. Such a female would not give a low female ratio, but since crossing over is known to occur between the arms of the double X at the four-strand stage, some of her eggs might then come to contain the postulated factors in both arms. Such eggs would be expected to give females that would give low ratios. This might explain the cropping up of low ratios from individuals when parents and grandparents did not show this result.

One mating of flies recorded in table 6 was also made for another generation. The result was F_3 yy ♀ 26:42 ♂. This female gave probably a low female ratio. Finally, a F_4 generation was reared in which sometimes vermilion males of the same generation and sometimes eosin males were used (table 7).

Table 7

F_4		F_4	
yy♀	♂	yy♀	♂
37	34	59	102
49	105	15	32
14	158	57	137
94	245	..	..

In all but the first case the ratios are exceptionally low in females. The fact that so many of these are low is probably due to the selection of parents from those stocks that had given exceptionally low ratios. Two explanations of these results are possible. First, low producing recessive genes were still present in some of the yy-females of the stock used, as postulated above, but present in a heterozygous condition. When crossing over takes place between the arms of the attached-X and such X's are retained in the egg, the latter would give rise to a low-producing-female, which would then become a low female producer. Second, that the stock, bred in mass, still retains a few low-producing females. In the mass-result their output is obscured by the general 1 : 1 ratio of the larger number of normal ratio females. When pair matings are made the presence of such individuals is revealed.

It was therefore important to determine whether the original stock still carried the gene in question in single or double dose. The experiment was made again in 1927 when several yy-stocks were still on hand. Males of low-female yy-stocks (41 ♀; 116 ♂ and 63 ♀ 188 ♂) were bred to attached-X (yy) females of stock not known to be giving low-female ratios. The yy-females came from stock with eosin singed males. Five cultures gave the following ratios:

yy ♀........	9	23	44	1	70
♂........	39	49	110	8	128

Here all the ratios are low. It was barely possible that the singed males were responsible for the result; hence, it became necessary to test the original (not low) yy stock both with eosin singed and with other males. The results are recorded in table 8.

From three of these stocks only normal ratios were obtained; in one other stock (yy ♀ by lz5 (yy) ♂) two of the females (bred to brothers) gave, possibly, low female ratios; one female at least bred to the eosin singed stock gave a very low ratio. Evidently the low ratios from the attached-X females were due to the presence of a factor or factors which in this stock give low-producing females. It follows that the ordinary attached-X stock gives rise at times to low producing female ratios and that the experiment made above to test the effect of the low female-producing male of the low female-producing stock was inadequate, because of the occasional presence of low-producing females in the ordinary yy-stock. It is known in fact that most of the stocks with attached-X's do occasionally give low female ratios.

TABLE 8

yy ♀ by w^esn(yy) ♂		yy ♀ by lz 5(yy) ♂		yy ♀ by dx(yy) ♂		yy ♀ by svr(yy) ♂		yy ♀ by B (yy) ♂	
♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
79	117	37	36	99	94	55	35	78	92
38	60	76	92	87	74	14	12	81	91
2	29[a]	65	50	53	51	58	66	64	75
47	64	44	68[a]	84	49	54	43	70	63
45	64	63	93[a]	44	60	38	20	87	95
45	66	..	..	70	92	65	63	18	13
55	78	..	..	115	80	83	77	103	101
79	74	..	..	..	..	57	59	65	83
17	28	..	..	..	..	...	..	83	97

ORDINARY FEMALES OF OTHER MUTANT STOCKS MATED TO MALES OF LOW-FEMALE-PRODUCING STOCK

If the condition for low-producing female ratios is connected with the attached X-chromosome, then, since the X of the males of these stocks never gets into the female line, nor comes from that line (except in the case of broken X's to be described below), there would be no reason to expect that the males of low-female producing stocks would transfer the character of the stock to the descendants of ordinary females, *i. e.*, to females whose X's are not attached. On the other hand, if the low ratio were due to the autosomes alone, then the effect might be transferred from the males of the low-producing lines in one or two generations to other lines with the normal sex chromosome relations. Only the first generation was reared, which would suffice if the factor were an autosomal dominant, but an F_2 generation would be necessary if the factor is recessive.

Four forked[5] virgin females were mated to 5 Bar males of low-female producing stock and gave 30 heterozygous Bar daughters and 24 forked sons. One vermilion female was bred to low Bar males and gave 49 ♀ : 35 vermilion ♂. An eosin cut vermilion female bred to low Bar males gave 6 ♀ :10 w^e ct v ♂. A yellow scute female bred to 7 low males gave 114 ♀ : 86 yellow scute ♂. An apricot forked female bred to low Bar male gave 158 ♀ : 114 w^e f ♂. A vermilion female was bred to a low male and gave 89 ♀ : 82 ♂.

These results indicate clearly that the males of the low-female-producing stock, when outbred to females of other stocks having normal sex ratios, do not change the normal ratio. The Y-chromosome of the father passes to the first generation daughters, hence even if it were a dominant agent in the result, its effect would show in the first generation offspring. This experiment was not followed to the second generation but in a repetition of the experiment this was carried out. The first generation offspring is given in table 9.

TABLE 9

♀	♂	♀	♂	♀	♂
(1) 87	95	(4) 112	85	24	29
80	82	79	86	58	37
24	16	79	62	82	72
49	32	82	60	24	29
64	69				
107	91	(5) 108	119	(6) 53	23
50	54	87	95	158	114
		80	82		
(2) 114	86	24	16	(7) 30	24
		49	32	50	43
(3) 113	119	64	69		
118	116	107	91	(8) 114	86
93	98	50	54	...	...
42	46	49	35	...	...
138	135	11	16	...	...
...	...	6	10	...	...
...	...	82	77	...	...

Vermilion eyed females gave the first seven broods in table 9, (1); a yellow scute female the eighth, (2); Star Dichæte Curly females the ninth to the thirteenth, (3); eosin females the fourteenth to seventeenth, (4); vermilion females, again, the 18th to the 33rd, (5); apricot forked females the 34th to the 35th, (6); forked females the 36th to the 37th, (7); a yellow scute female again, the 38th, (8).

These results show again that there is no decrease in the number of daughters in the first generation. With one exception (52 : 23) the differences observed seem to be normal fluctuations. In the one exception a sex-linked lethal may have been present, and, since the sex ratio is in the reverse direction, it does not affect the general conclusion.

A few second generations were bred from the F_1 females and males of table 9. The results are given in table 10. There is no evidence that the P_1 male has introduced a change in the F_2 sex ratio.

TABLE 10

♀	♂	♀	♂
80	59	48	41
53	50	28	19
93	131	28	32
38	40	28	21

A repetition of the experiment in 1927 gave the same results, namely, the failure to transmit the low-producing character through the males of the low-producing line to other mutant stocks with unattached X-chromosomes.

RELATION OF CHROMOSOME-II AND III TO THE ABERRANT SEX RATIOS OF ATTACHED-X STOCK

The usual procedure in locating modifying recessive genes in specific chromosomes in *Drosophila* was utilized in an attempt to discover whether there are modifying genes in the second and third chromosome of the low-female-producing stock that are responsible for, or contributory to, these ratios. Males of Star Curly Dichæte stock (S Cy D) carrying three dominant genes, two (S and Cy) in the second and one (D) in the third chromosome (diagram 1), were crossed to attached-X yellow females of low-female-producing lines with the following results:

	yy ♀	♂
S Cy D ♂ by yy ♀ (28 ♀ : 109 ♂) (23 ♀ : 210 ♂) =	20	85
S Cy D ♂ by yy ♀ (19 ♀ : 113 ♂) =	2	12
S Cy D ♂ by yy ♀ (28 ♀ : 221 ♂) (22 ♀ : 210 ♂) =	2	139

It may appear, at first sight, that these low female ratios obtained in F_1 show that if autosomal intensifiers are present they must be dominant since the introduction of one set of autosomes, derived from the Star Curly Dichæte stock, did not change the aberrant ratios, but if the postulated autosomal genes in the low-female-producing stock should produce their effect on the egg *before reduction* it would be necessary to carry the experiment through further generations in order to determine whether the postulated intensifiers are recessives. But this test cannot be made in this way by the use of such dominants as Curly and Dichæte which are lethal when homozygous; for, in every successive generation a second and a third chromosome (the mates of Curly and Dichæte) may still be present. The F_1 Curly Dichæte males were picked out *and bred to attached-X, yy-females of stock (giving normal sex ratios)*. The female offspring of

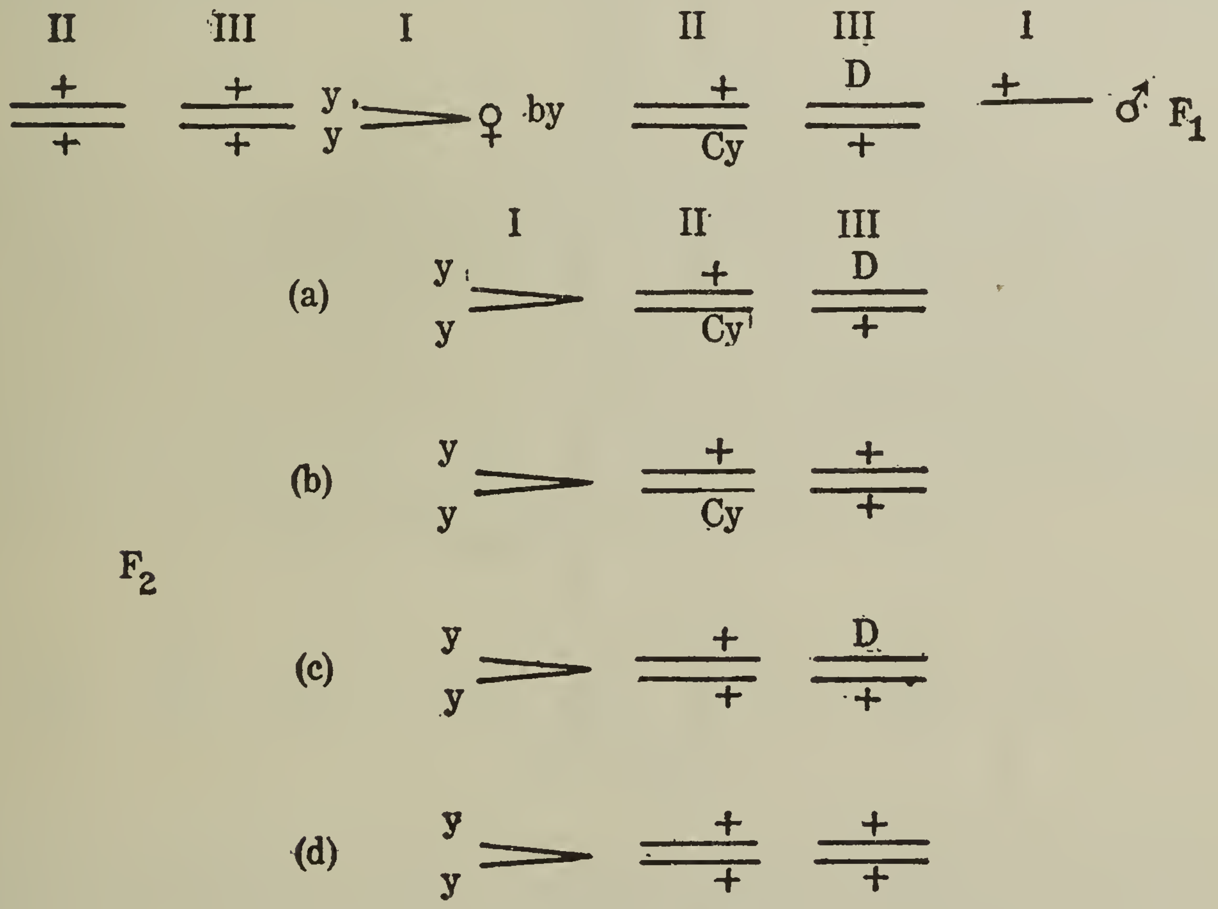


Diagram 1

this mating each carrying attached-X chromosomes will be of various kinds: (diagram 1, (a), (b), (c), (d)). (a) some will be yy Cy D and have both one second (Cy) and one third (D) chromosome and the yy of the normal ratio stock; (b) some will be yy Cy and will have one second chromosome (Cy) and the original third pair of chromosomes of the low stock and the yy of the normal ratio stock; (c) some will be yy D and will have one dichæte third chromosome (D), and the second pair of chromosomes of the low stock and the yy of the normal ratio stock; and (d) some will be yy and have neither the Curly nor the Dichæte chromosome and the yy of the normal ratio stock. These four kinds of F_1 females were out-bred to males of ordinary stocks, such as vermilion and eosin cut, in order to find out whether one kind of female gives different sex ratios from the others, depending on their autosomal make-up. The results are given in table 11.

TABLE 11

	yy ♀	v ♂
F_1 Cy D yy ♀	=29	44
	29	34
	17	26
	35	77^{b}
	51	53
	46	78
F_1 Cy yy ♀	=25	45
	36	71^{b}
	23	27
	68	96+(1 gray ♀, XXX)
	22	22
	19	33
	12	20^{b}
F_1 D yy ♀	=32	37
	42	48
	38	57
	25	37
	56	51+(2 gray ♀, XXX)
	10	27^{b}
	21	18
	20	22
	14	12
	17	27
	18	7
	13	16
F_1 yy ♀	=45	48
	31	54
	44	55
	55	73
	26	26
	57	58

There are no noticeable differences in the females (the mother was from not-low stock). In many broods there is a slight excess of males but this may be due to the lower viability of yellow flies. In addition there are several broods (marked with ^{b}) in which there are nearly twice as many males as females.[1] Since the attached X's in F_2 came from stock (not low) these ratios are probably due either (1) to the introduction of new autosomes, or (2) to the not-low yy-stock still carrying a factor in the attached-X's. The former explanation (1) would account for some of the low ratios that are not extremely low and if so the autosomal gene is partly dominant (affecting to some extent the double X (not low) female of stock), but the 1 to 2 ratios are more probably due to the unexpected presence of low-producing-females, since other tests have shown that low females do appear in the attached-X stock.

The experiment gives a positive answer to one of the questions involved, namely, that the autosomes of the low-producing line in combination with the attached-X's (not low female producing) does not give the extremely low female ratios; and since as other evidence has shown the not-low stock still contained females that are low female producing, the low ratio broods were probably due to this condition.

LOW FEMALE PRODUCERS WITH ATTACHED X's BRED TO STAR DICHÆTE MALES AND LATER GENERATIONS

Six F_1 males and six F_1 females obtained by mating S Cy D ♂ (stock) to low yy ♀ (28:221; 22:210) were mated in pairs (diagram 2) and gave the following ratios:

yy ♀	6	11	2	10	5	11
♂	177	251	162	88	70	63

Exceptionally low ratios appeared in every one of the six combinations. Each of the F_1 mothers had a foreign chromosome of the second and third pair (II and III) and the attached X's of the low female line. As before it follows either that the attached X's alone are responsible for the exceptional ratios, or else, since each had also one of the second and one of the third chromosomes derived from the grandmother, one (or both) of these carries a dominant modifier which, taken in connection with the attached X's of the low strain, gives the exceptionally low female ratios.

$$F_1 \quad \overset{\text{II}}{\frac{\text{Cy}}{+}} \quad \overset{\text{III}}{\frac{\text{D}}{+}} \quad \overset{\text{I}}{\begin{matrix}\text{y}\\ \text{y}\end{matrix}} \succ\!\!\!\longrightarrow ♀ \text{ by } \overset{\text{II}}{\frac{\text{S}}{+}} \quad \overset{\text{III}}{\frac{\text{D}}{+}} \quad \overset{\text{I}}{\frac{+}{}} ♂$$

DIAGRAM 2

In a second experiment yy-females of low-female stock were first mated to Star Curly Dichæte males. The F_1 daughters were yy Cy D, yy Cy, yy S D, and yy S. The attached X's of the mother are here those of the low-female stock. Through an error these four kinds of F_1 females were first mated to their brothers that were not-Star, not-Curly, not-Dichæte. The result will be given later. When this mistake was realized the old females were mated a second time to dominant Lobe-eyed males. The data for the dominant Lobe flies could at least be used. The following results were obtained (table 12).

---

[1] There is one brood in which the reverse ratio occurs, namely, 18 females and 7 males.

TABLE 12

	yy ♀	yy Lobe ♀	♂	Lobe ♂
S D yy ♀..........	5	0	24	45
	3	0	23	46
	0	6	2	18
	0	0	3	1
	0	0	17	65
S yy ♀............	0	0	4	7
	3	0	9	0
Cy yy ♀...........	3	0	33	19
	2	2	28	50
Cy D yy ♀.........	0	16	0	101
	0	0	3	23
	2	0	15	11
	0	8	0	40
	14	5	41	39

The result in every case was the same; an exceptionally low female ratio—as low as in the original low-female-producing stock. The result shows that both the second chromosomes and both the third chromosomes are not essential to the production of exceptionally low ratios.

As stated above, the F_1 yy-daughters had been by mistake first mated to their brothers that were not-Star, not-Curly, not-Dichæte. The result is given in table 13.

TABLE 13

	yy ♀	♂
S yy ♀ by sib S ♂ =	0	21
	1	25
	13	94
	1	15
	1	11
S D yy ♀ by sib S ♂ =	0	6
	3	35
	24	149
Cy yy ♀ by sib S ♂ =	1	19
	0	9
	46	69
C D yy ♀ by sib S ♂ =	32	60
	4	26
	0	27
	3	38
	1	43
	0	4
	1	8

Now these brothers had an X-chromosome from their father (the same as that in their grandfather) and two second chromosomes that may have come, one or both, from either grandparent, and similarly for the third chromosome. Hence the experiment is not discriminative because in some cases at least the original second and third chromosomes may have recombined. Nevertheless the results are probably significant since in all cases low female ratios were obtained and in nearly all cases exceptionally low female ratios. The result is essentially the same as in the second mating (given above) of the same females.

RECIPROCAL CROSS OF STAR CURLY DICHAETE FEMALE BY "LOW" BAR MALE

A reciprocal cross was made five times in which a Bar male (of low-female producing attached-X stock) was mated to a Star Curly Dichæte female with the following results. The low ratios of several preceding generations of these males is recorded in parentheses.

	♀	♂
S Cy D ♀ by Bar ♂ (28-109; 23-40) =	70	54
Do (8-165; 24-61) =	93	81
Do (7-103; 6-31; 18-130; 9-107) =	85	101
Do (4-80; 7-44; 17-130; 8-107) =	46	44
Do (5-70; 8-48) =	64	80

The absence of low female ratios indicates that the autosomes and the Bar X of the father (from low-producing-females) carry no dominant factors (in conjunction with the X of the S Cy D stock) that gives exceptional ratios. This experiment is essentially the same as that described in an earlier experiment except that Star Curly Dichæte females were used from the same stock that had supplied the males of the last experiment.

BACK-CROSS OF F_1 CURLY HAIRLESS MALES TO LOW-FEMALE PRODUCERS OF ATTACHED-X STOCK

This experiment made a year later involved two dominants, namely, Curly in Chromosome-II and Hairless in Chromosome-III. Low, female-producing, yy females, mated singly to Cy H males, gave in the first generation the following results:

TABLE 14

yy ♀	♂	yy ♀	♂	yy ♀	♂
4	52	53	69	43	125
19	46	18	117	51	99
52	70	12	20	10	89
25	63	0	12	13	51
6	65	10	24	27	86
5	15	69	179	26	82
..	..	..	..	34	73

The ratios are typical low-stock ratios. The F_1 males and females are of four kinds, and in one culture they were separated according to their classes, as below:

yy Females				Males			
Cy H	Cy	H	+	Cy H	Cy	H	+
18	17	23	15	28	19	31	19

The sex ratio here was 73 and 97, which is not as low as most of the ratios of table 14. All classes are represented both in the females and males and in fairly equal numbers for each sex.

Some of the F_1, Cy H males were next mated to yy low-female producing females and gave the following ratios:

TABLE 15

yy ♀	♂	yy ♀	♂	yy ♀	♂
14	26	37	49	21	40
11	29	21	20	22	30
25	66	22	30	14	47
25	29	40	47	35	43
19	16	33	68	25	29

In ten cases the classes were separated and gave the following results:

TABLE 16

yy Females				Males			
Cy H	Cy	H	+	Cy H	Cy	H	+
11	5	15	5	15	9	4	6
4	1	18	7	7	3	16	16
12	2	13	5	14	12	11	10
12	8	19	3	18	8	22	9
2	4	1	5	5	2	4	7
15	17	17	3	10	21	14	9
14	3	5	5	2	3	3	4
22	15	23	6	32	26	27	22
8	2	12	4	10	3	14	13
10	7	9	8	17	8	12	11
110	64	132	51	130	95	127	107

There were in all, 353 females and 459 males which unfortunately is not a very striking departure from normal. The Curly and normal females are

TABLE 17

yy Females				Males			
Cy H	Cy	H	+	Cy H	Cy	H	+
1	3	2	1	5	10	12	8
15	12	12	3	23	6	23	8
24	19	16	11	26	28	12	14
1	1	0	0	1	0	2	2
25	38	17	9	18	16	16	16
16	7	15	8	23	13	10	11
82	80	62	32	96	73	75	59

about half as numerous as the Curly Hairless and Hairless. This might seem to mean that a modifier for low ratios is present in the third chromosome, especially as the same classes in the males do not run significantly lower than the other two classes.

In another series of the same sort the F_1 Curly Hairless males were bred to attached-X, yellow, low-producing females (table 17).

The sex ratio here, as before, is not very low (256 ♀ to 303 ♂). In the females the Curly class is not low, but that of the normals is less than half the others. Some of the F_1 females were mated (in pairs) to Bar males of the low-female producing stock, with the following results:

TABLE 18

F_2 yy Cy H ♀ by B ♂			F_2 yy Cy ♀ by B ♂				F_2 yy H ♀ by B ♂			F_2 yy + by B ♂			
yy ♀	B ♂	het. B ♀	yy ♀	B ♀	het. B ♀	y ♂	yy ♀	B ♀	het. B ♀	yy ♀	B ♂	het. B ♀	y ♂
87	141	....	56	99	....	1	60	139	....	94	134	....	....
106	247	....	65	145	1	....	43	112	....	62	175	1	....
125	222	1	66	85	....	....	69	130	....	92	144	1	....
170	229	(N ♂ 6)	99	144	....	....	117	241	....	94	219	....	....
161	220	....	111	197	....	....	112	157	....	42	83	....	....
72	131	1	35	97	1	....	65	95	1	89	129	1	....
80	154	....	43	77	....	....	....	....	....	71	103	....	....
147	313	....	68	196	....	....	....	....	....	82	70	....	....
125	164	1	....	....	....	....	....	....	....	30	177	....	....
43	109	....	....	....	....	....	....	....	....	100	83	....	....
....	....	....	....	....	....	....	....	....	....	72	85	3	....
....	....	....	....	....	....	....	....	....	....	102	89	....	1
....	....	....	....	....	....	....	....	....	....	88	100	....	....
....	....	....	....	....	....	....	....	....	....	18	92	....	....
....	....	....	....	....	....	....	....	....	....	23	32	1	....

With only a few exceptions low-female ratios occurred in all the cultures. None of them, however, are extremely low. There are no evident differences between the four kinds of F_1 females that were here tested.

In addition to the matings that gave the results in table 17 there were a few others in which F_1 males and females of different kinds were paired. The results were as follows:

			yy ♀	♂
F_1 yy Cy H ♀ by F_1 Cy H ♂	(both out of yy (low) ♀ by Cy H ♂)		19	45
F_1 yy Cy ♀ by F_1 Cy ♂	Do.	(1 ♀ :2 ♂)	39	49
Do.	Do.		75	79
Do.	Do.	(14 ♀ :29 ♂)	18	26
F_1 yy + ♀ by F_1 Cy H ♂	Do.		31	86
Do.	Do.		13	60

The data give no further information than that already obtained. In all but one case low ratios appear to be present.

Several combinations were made from F_3 brothers and sisters from the flies recorded in table 18 in order to see whether by continued selection from the lower-female-producing stocks, still lower ratios could again be obtained. The results recorded below show positively that ratios as low on the average as those of the original low-producing stock were recovered when brother-sister matings were made in later generations (table 19).

TABLE 19

	yy♀	♂	yy♀	♂	yy ♀	♂	y♂	N♀
F_3 yy ♀ by Cy H ♂	76	95	40	36	6	11		
Do.	12	105	56	85	17	62		
Do.	28	57	18	16	53	43		
F_4 yy Cy H ♀ by Cy H ♂ (9♀:40♂)	1	5						
Do.	7	18						
F_4 yy Cy H ♀ by H ♂ (6♀:73♂)	20	42						
F_4 yy H ♀ by Cy H ♂	12	54					1	1
Do.	38	61						
Do.	50	92						
Do.	17	24						
F_4 yy Cy ♀ by H♂	12	59						
F_5 yy Cy H ♀ by Cy H ♂ (12–59; 9–40)	2	24						
F_4 yy H ♀ by H ♂ (36–75)	28	59						
Do. (7–41; 6–20)	27	49						
Do. (18–48; 36–75)	53	66						
Do. (20–42)	36	129						
Do. (8–40)	30	24						
F_5 yy H ♀ by H♂ (4–22)	29	34					1	
F_3 Cy H ♀ by yy high	76	95						
	12	105						
	28	57						
	46	36						
	56	85						
	18	16						
	6	11						
	17	62						
	53	43						
F_4 yy Cy H ♀ by Cy H ♂ (9–40)	1	5						
yy Cy H ♀ by H ♂ (9–40)	7	18						
yy H ♀ by Cy H ♂ (2–58)	12	54					1	1
yy H ♀ by H ♂ (6–37)	38	61						
yy H ♀ by H ♂ (6–73)	50	92						
yy H ♀ by H ♂ (6–37)	17	24						
yy Cy ♀ by H ♂ (9–40)	12	59						
yy Cy H ♀ by H ♂ (6–73)	20	42						
F_5 H ♀ by H ♂ (4–22)	29	34						
F_5 H♀ by H♂ (4–22)	4	35					1	
Cy H ♀ by Cy H ♂ (2–8; 9–40)	30	24						
yy Cy ♀ by Cy H ♂ (31–102; 32–70)	7	21						
F_6 yy Cy H ♀ by Cy H ♂ (18–48; 36–75)	53	66						
(7–41; 6–20)	27	49						
(4–34)	19	69						
(4–34)	3	22						
yy H ♀ by H ♂ (18–48; 36–75)	51	67						
yy H ♀ by H ♂ (7–41; 6–20)	10	28						
yy Cy H ♀ by Cy ♂ (7–41)	43	85						
yy Cy H ♀ by Cy H ♂ (4–31)	30	91						
F_7 yy Cy H ♀ by Cy H ♂ (19–67; 4–34)	28	123						
Do. (8–35)	11	66						
yy H ♀ by Cy H ♂ (4–24; 2–58)	8	19						

The results show that the very low ratios again appear after selection in these stocks that contain the attached X's of low-female producing origin, even when one or both of the second and third autosomes are present. But since crossing over had not been suppressed there is the probability that the chromosome marked by Curly and Hairless would have come to contain modifiers, if such are present in the opposite chromosome derived from the low-female producing stock.

BACK CROSS OF F_1 CURLY DEFORMED ATTACHED-X FEMALES TO WILD-TYPE MALES

A complete disproof of the possible view that the low ratios are due to dominant modifiers in the second or third chromosome was later made manifest by the use of a stock that contained the dominant genes Curly (II) Deformed Eye (III) and non-crossover factors called C. The presence of the latter almost completely suppresses crossing over in the second and third chromosomes. A low-female-producing yy-female was mated to a male of that stock. The daughters that were Curly Deformed were then mated to a wild-type male. Diagram 3, page 124, of P_1, F_1, and F_2 gives the combinations of the chromosomes in this case.

TABLE 20

F_1 Curly Deformed C yy ♀ by wild ♂								
		yy ♀	Gray ♂		yy ♀	Gray ♂	yy ♀	Gray ♂
F_2.............		49	76		44	61	114	170
		79	55		70	81	33	59
		14	43		3	17	57	59
		32	70		138	179	69	80
		90	94		153	173	115	178
		27	33		42	47	25	70
		116	156		50	67	....	....
F_2 Curly Deformed C yy ♀ by wild ♂								
F_3.............		26	24		23	25	22	25
		11	16		38	41	28	32
		18	35		41	190	15	90
		30	78		33	44	56	79
		5	11		42	81	67	73
		18	34		25	69	5	134*
		23	30		12	25	45	69
		70	111		17	30	..	..
F_3.............	(60–70)†	6	44	(29–54)	1	14	..	..
	(72–116)	3	51	(13–22)	2	52	..	..
	(72–116)	4	81	(42–49)	99	128	..	..
	(72–116)	11	40	(66–80)	15	31	..	..
	(66–74)	48	83	(66–80)	50	130	..	..
		36	53	(28–163)	12	30	..	..
		5	58		..	..	..	..

* Plus 1 gray ♀. † Complete record of preceding generations.

As a result of the F_1 mating the F_2 attached yy females that are Curly Deformed have as mates of their two autosomes a second and a third chromosome from the F_1 wild-type male as the other member. In other words the second and third chromosomes of the original yy stock have been replaced. If then the F_2 females still give low ratios, the second and the third pairs of chromosomes are not responsible for the result. The outcome shows that low ratios still appear (table 20).

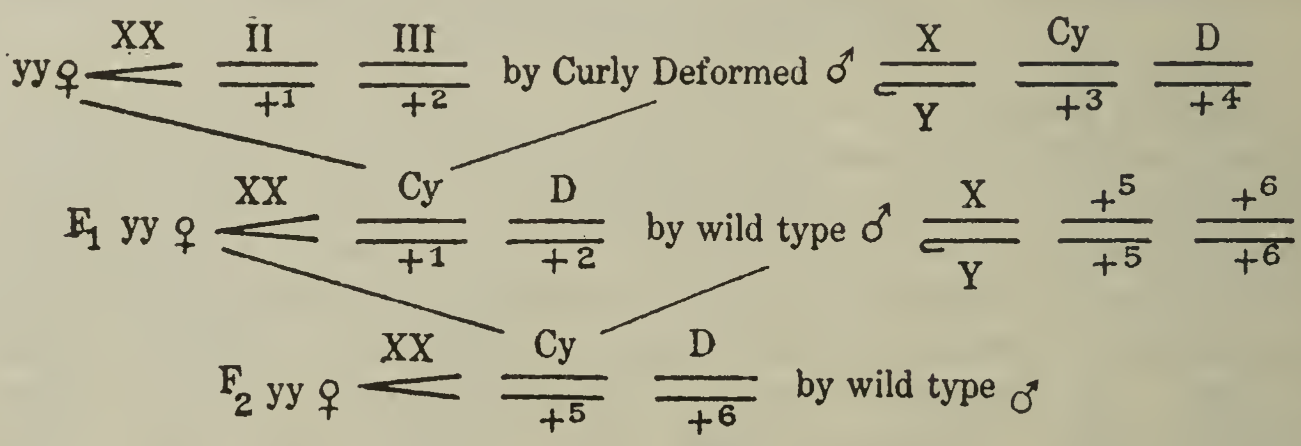


Diagram 3

In the course of the experiment a few other matings were made between other combinations of F_1 and F_2, besides those described above. These may be given although they add little further to the evidence (table 21).

Table 21

			yy ♀	Wild Type ♂
F_1 yy ♀	by F_1 Wild Type ♂	=	6	20
F_1 yy Curly ♀	by F_1 Wild Type ♂	=	31	109
F_1 yy Deformed ♀	by F_1 Deformed ♂	=	16	61
F_1 yy Curly Deformed ♀	by F_1 Curly Deformed ♂	=	89	202
F_1 yy Curly ♀	by F_1 Curly ♂	=	8	89
F_2 yy Curly ♀	by F_2 Wild Type ♂	=	28	186
F_2 yy Curly ♀	by F_2 Wild Type ♂	=	94	130
F_2 yy Wild Type ♀	by F_2 Wild Type ♂	=	73	318
F_2 yy Wild Type ♀	by F_2 Wild Type ♂	=	75	133
F_2 yy Curly Deformed ♀	by F_2 Curly Deformed ♂	=	5	61
F_2 yy Curly Deformed ♀	by F_2 Curly Deformed ♂	=	12	45
F_2 yy Curly Deformed ♀	by F_2 Curly Deformed ♂	=	6	47
F_2 yy Wild Type ♀	by F_2 Curly ♂	=	15	102
F_2 yy Wild Type ♀	by F_2 Curly ♂	=	13	55

	Curly y ♀	y ♀	Curly Wild Type ♂	Wild Type ♂	y ♂
F_2 yy Curly ♀ by F_2 Curly ♂ =	2	1	21	9	1

In the F_1 crosses given above the original second and third chromosomes that were present in the F_1 female (before polar body elimination) may or may not have been replaced in the zygote. Nevertheless all the broods were very low in females. Similarly in the F_2 females that were not themselves Curly Deformed. Yet the latter gave the same ratios (low in females) as did the former. If, as the other evidence shows, the second and third chromosomes are not responsible either for the main results or for the variations in the ratios the latter results are expected.

Test for Chromosome-IV

In two generations the fourth pair of chromosomes of the high female-producing stock was exchanged for a new pair of chromosomes bearing eyeless.

The yellow attached-X females (48–87, 72–116) were bred to eyeless^2 males and gave 79 yellow females and 172 "gray" males.

Six F_1 yellow females were back-crossed to eyeless^2 (stock) males and gave the following results. The failure of many of the eyeless flies to show the small eye is obvious:

yy ♀	yy-eyeless ♀	N ♂	eyeless ♂
25	6	60	13
37	1	183	3
60	1	100	0 Gray ♀ 1
109	38	202	101
3	2	29	0
22	15	26	12

Eyeless females and males were mated in pairs and gave the following eyeless offspring. The sex ratios are those characteristic of the original low-female-producing stock.

F_2 Eyeless

yy ♀	N ♂	yy ♀	N ♂	yy ♀	N ♂	yy ♀	N ♂
93	163	63	145	138	222	35	71
52	186	82	144	81	234	24	50
24	106	61	175	86	115	35	71
126	231	110	150	47	63	24	50
42	119	31	51	12	56	40	46
..	..	..	..	8	24	34	67
..	..	..	..	18	50	21	54
..	..	..	..	..	..	22	55

In addition to the records in the last table two cultures gave one gray female which must have been either triploid or else derived from a broken yellow X by a normal gray X from the male.

Three F_3 cultures of eyeless were recorded and one F_4 as follows:

Eyeless

	yy ♀	N ♂		yy ♀	N ♂
F_3..........	51	71	F_4..........	67	254
	0	22			
	39	87			

The results show that the fourth chromosomes may be replaced by those from eyeless stock without affecting the low female ratios, thus excluding the fourth chromosome from the case.

ATTACHED-X WITH THE GENE FOR YELLOW IN EACH X, GIVING RISE TO GRAY ATTACHED-X FEMALES

A gray-winged daughter appeared from a mating of a yy attached-X female to a gray Bar male. She was bred and produced some gray daughters which in turn were bred, and from some of these both gray and yellow daughters were produced. At the time these results were not understood, but parallel cases worked out by Sturtevant, Bridges, Anderson and L. V. Morgan have given the solution. The first female must have been a triploid with one double yellow (yy) X-chromosome, and another X that had a gray-producing, wild allelòmorphic gene. One gray gene dominates two

Table 22

	n n ♀	w^e ct v ♂	
n n ♀ by w^e ct v . ♂	80	57	
	41	39	
	132	133	
	n n ♀	w^e v f^5 ♂	
n n ♀ by w^e v f^5 ♂ . .	65	53	
	n n ♀	B ♂	
n n ♀ by B ♂......	16	81	
	10	58	
	13	109	
	15	35	
	24	30	
	58	98	
	19	20	
	14	75	
	29	100	
	41	113	
	14	24	
	28	76	
	n n ♀	ct ♂	
n n ♀ by ct ♂......	89	95	
	50	54	
	n n ♀	yy ♀	B ♂
n n ♀ by B ♂......	10	12	32
	52	12	81
	70	14	179
	33	9	41
	21	8	44
	28	1	48
	28	8	44
	44	9	61
	42	9	49
	19	7	20
	12	9	15
	52	16	75
	21	2	31
	7	10	51
	5	6	16

yellows. Crossing over is known to occur in such triploid females between the attached-X's and the unattached X, with the result that some of the daughters with attached-X's will have substituted the allelomorphic normal gene for the yellow in one arm of the attached-X chromosomes. Crossing over is now known to take place at the four-strand stage between one X of one strand of one arm and the other X of the other strand of the other arm. Some of the daughters of the triploid will be diploid, having an attached-X with a yellow gene in one arm and a gray gene in the other. In these females crossing over between the arms of the attached-X's will take place at times at the four-strand stage of such a kind that the yellow gene of one strand of one arm will be exchanged for the normal gray gene of the other strand of the other arm, giving rise to a female that has the normal gray gene in both arms. The former kind of female heterozygous for gray and yellow will, when mated to a gray male, produce both gray attached-X daughters (heterozygous for yellow) and yellow attached-X daughters arising from a second recrossing over. The latter kind of female (homozygous for gray) will produce only gray attached-X daughters.

In both cases the sons will be patroclinous for the X-chromosome.

The data recorded in table 22 give the two kinds of results from diploid gray females derived as above from a triploid ancestor.

In the middle set in the above table nearly all of the ratios are "low," but in none of the others. This difference must be due to the different compositions of the attached-X's resulting from crossing over in the triploid ancestor between the attached yy chromosome and the introduced X of the male.

SEX RATIOS IN "BROKEN-X" FEMALES

It did not seem probable, at first, that the union of two X-chromosomes could in itself be responsible for low-female ratios, because, in the ordinary stock of attached-X, low-female ratios were not as a rule present. In order to find out whether these X's carried in themselves any factors contributing to the peculiar ratios, a new stock was made up derived from "broken" yellow males that occasionally appear by the separation of attached-X chromosomes. The main requirement in such an experiment, which includes at least two generations, is that crossing over in the F_1 females does not take place. This is easily accomplished by utilizing a stock containing factors for non-crossing over. We have such a stock in which one X-chromosome of the female carries a gene for fused and the other X the gene for Cl (non-crossing over) and for Bar. The experiment took place in several steps (see diagram 4).

1. A female $\frac{\text{fu}}{\text{Cl B}}$ was crossed to a broken yellow male derived from a low-female-producing stock. The heterozygous Bar daughters resulting from this mating had the Cl B chromosome of the mother and the broken yellow X-chromosome of the father. Crossing over was prevented by the presence of the Cl factor. This female was then mated to a broken yellow male that had been obtained as indicated below (2).

2. The first male that was used in (1) had been simultaneously mated to an attached-X female of the low-female line. His sons received the broken X of the father and contained also the same autosomal complex as the males of the low-producing stock.

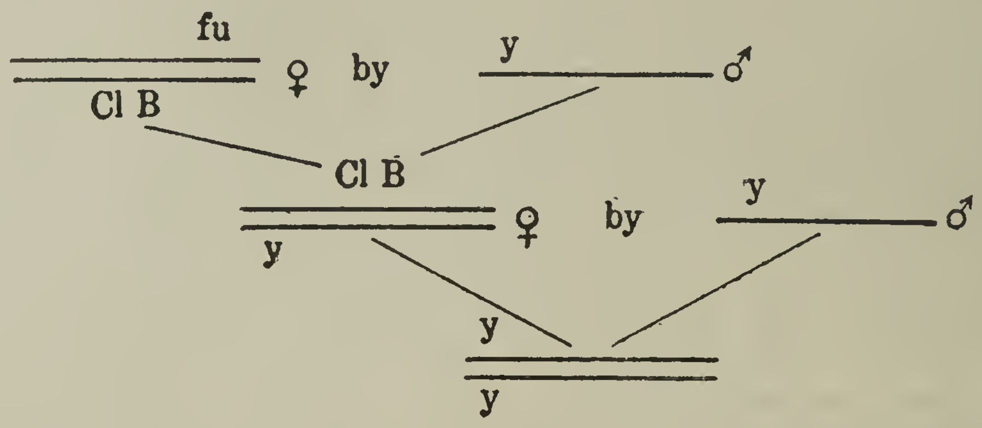


Diagram 4

3. The females from (1) and the males from (2) were, as stated above, then mated. All the yellow daughters contain two of the broken yellow chromosomes which are the lineal descendants (without any cross over complications) of those from the original broken yellow grandfather.

4. These "broken yellow" females bred to their yellow brothers give the required material to test the problem. It will be noted that their autosomal composition is expected to be mixed inasmuch as some of these females may have autosomes derived from their grandmother; but amongst them there may be some that happen to receive the original set of autosomes. Selections from pair matings will then be expected in time to sort out these females from the others, and if the presence of the autosomes of the low-female-producing stock increases the chance of very low female ratios, the derived, single-yellow stock will be identical genetically with the original double yellow stock.

A broken yellow male was mated to a fu Bar female, whose formula is $\frac{\text{fu}}{\text{Cl B}}$. Some of the heterozygous Bar daughters (F_1) were mated to broken yellow males (see diagram 4), and gave the following ratios:

het. B ♀ and y ♀.......	34	52	15	31	27
y ♂..............	21	17	6	7	10

Incidentally, it is significant to note that one (broken) yellow chromosome in the female does not lower the female ratio, which means, that if the low factor is still present in the broken-X, it is recessive. The ratio of two females to one male is here due to the Cl B lethal chromosome that kills half of the males.

Some of the yellow females (in pairs) were bred to their yellow brothers (see diagram 4) and gave:

y ♀....................	103	138	124	82
y ♂....................	142	202	126	123

Two and probably three of these sets gave indications of a low female ratio that was, however, not extreme. From these and from their offspring many pairs were made up. The results are given below. The ratios of the preceding generation are indicated in brackets.

	y♀	y♂
y ♀ by y ♂ (16♀ –14♂)..	140	125
	120	159
	86	118
	43	88
	53	82
	92	124
	116	117
(45♀ –78♂)..	42	46
	92	103
	90	158
	100	108
	67	96
(10♀ –17♂)..	109	157
	41	40

Some of these ratios seem to be 1 : 1; some of them seem to show an excess of males; one at least is 1:2 but others do not have as low female ratios as this. From several of these sets, usually where the lower ratios had appeared, further matings were made (table 23). It is not possible to refer these to the ratios above because the matings were made before the cultures had run out and the final ratio was not then known. The ratios present at the time the selection was made is indicated (in parentheses) in some cases for one, in others for the two preceding generations.

Since, in a few cases, 1 : 2 ratios appeared, the conclusion may seem to follow that a recessive factor was present in the detached (broken) X that was responsible for the result, acting as semi-lethal; but if so, why are most of the ratios normal? Since all the X-chromosomes are identical, it is not apparent why the results are not uniform unless indeed the few cases of apparently low ratios were merely accidental. It is true that the X-chromosome of the male is now transferred in each generation to the daughters (and not retained in the male line as in the attached-X stock), but the X of the male has here had the same origin as the other X's and should carry the same genes. It might be argued, however, that the new detached yellow-bearing chromosome in the male may also affect his viability, although not to the same extent as it does in the female.

The situation with respect to the broken yellow males is probably not so simple as the preceding analysis implies, even if the low female ratios of the stock from which they came were due to two recessive factors one in each arm of the double X. In the first place the effect is only semi-lethal destroying only some of the females. Now, if the double X-chromosome becomes separated, and each arm carries the semi-lethal gene in question, it might be expected, as stated above, to produce a semi-lethal effect on the males where one X-chromosome is present. On the other hand, the effect on the males might not be the same as on the females. If it were the same, then, when the detached yellow stock was made up the sex ratio might be expected to be 1 : 1, if both sexes are affected to the same extent. Whether they were so affected could only be found out by making suitable tests. As a matter of fact, the first generation gave both equal and unequal ratios. If this were a typical result it is not obvious why the ratio should ever be changed by selection in a definite direction unless other factors were introduced. This suggests a further consideration, namely, whether all the broken yellow males are alike. It is possible that in breaking apart, the division might not take place at exactly the same point at which union originally occurred, hence there might be more than one kind of broken yellow X. If, then, some lines descended from one son of a female and other lines from other sons, the result might be different. On the other hand if the chromosome had not broken very near to the attachment point an X that lacked a part would probably not produce a viable male, or if it did defects in such a male would be expected. If a broken X contained a piece of its partner it too would not be expected to give normal results.

Table 23

Detached-X y ♀ by y ♂			Detached-X y ♀ by y ♂		
	y♀	y♂		y♀	y♂
(36–54)	88	130	(125–173)	23	33
(76–141)	27	21	(3–21)	38	44
(19–39)	52	50	(59–123)	129	129
(51–96)	35	77[a]	(5–10) (27–52)	23	22
(76–141)	93	142	(47–79) (68–123)	9	14
(68–123) (5–35)	35	34	(5–23) (95–195)	0	0
(76–141)	58	78	(76–140)	129	116
(95–175)	32	29	(51–101)	114	149
(53–105)	39	38	(76–141)	51	100[a]
(23–43)	60	67	(13–70) (1–14)	1	10[a]
(51–96)	69	90	(30–43) (36–54)	61	69
(5–31)	66	71	(30–43) (36–54)	33	43
(27–52)	73	71	(5–10) (27–52)	26	19
(16–14)	6	47[a]	(92–188)	118	198
(27–52)	33	35	(30–43) (36–54)	30	41
(36–54)	80	92	(46–102)	75	102
old dupl	54	116	(5–10) (27–52)	90	104
(53–105)	42	51	(92–156) (63–127)	34	20
(53–105)	125	173	(84–114)	26	37
(8–14)	58	282[a]	(3–21)	55	69
(68–123) (5–35)	30	38	(3–21)	64	74
(15–30)	28	32	(3–21)	45	56
(5–31)	46	39	(84–114)	37	64[a]
(95–175)	14	10	(5–10) (27–52)	43	42
"	13	30	(84–114)	32	58
"	51	96	(14–63)	61	74
"	29	24	(9–43) (95–195)	118	171
"	123	157	(4–17) (3–20)	33	42
(3–14)	100	143	(91–186)	56	52

The formation of a stock from the broken yellow chromosome does not then necessarily mean that it should always be expected to give low female ratios. In fact the result might be reversed if the effect of the semi-lethal gene or genes was greater on the male than on the female. As a matter of fact low-female-producing stocks were obtained with yellow males having the same X as that in the females. It follows that the semi-lethal gene or genes does not affect the male to the same extent as it does the female.

Unfortunately at the time that the first experiments were made it was not realized that broken yellow males, even from the same female, might be different, but since, in the experiment quoted, in which broken males were used and low ratios produced, the results do show that separate X's may give the same low ratios as attached X's.

A repetition of the experiment was carried out in 1927–1928. Females from the low producing stock were bred to wild males. A few wild-type females were obtained which are expected if the X's of a female had become detached. It was found, however, that these females were triploids, hence the X's had not become detached. One wild-type X dominates two yellow bearing X's. Yellow males derived from these females may be due to crossing over in the female triploid between the free X and the attached X's, hence they may lack the postulated genes and be useless as tests.

In two other cases, however, yellow males were obtained when a low-producing attached-X female was bred to a wild-type male. These new yellows were then used to make a new yellow stock by the procedure already described. This stock gave almost exclusively 1 : 1 ratios. There were sometimes a few more males than females, but the characteristic ratios did not appear. The evidence goes to show that the detached X's do not always give the characteristic results.

In order to see if any visible differences are present in the mode of attachment of the X's in the low-female producing strains a large number of sections of ovaries were prepared and metaphase plates studied and drawn. These plates show the attached-X's but the picture is the same as in the chromosome groups of ordinary attached-X stock. The evidence is, however, negative, for differences, if present, might be too slight to be revealed in the chromosomes at this stage.

There is another alternative possibility remaining to be considered that may account for the low ratios that occur at times in the original stock, as well as account for the return change in the low stock to a normal ratio. If some shifting of the mode of attachment of the X's occurs which leads more frequently to their elimination from the egg at the time when the polar bodies are produced, a formal explanation may be offered. This would decrease the proportion of eggs that produce females, and since such of the eggs as are entered by an X-bearing sperm will produce males, the number of the latter is automatically increased. The following diagram (diagram 5) illustrates how the change in ratio takes place on this assumption.

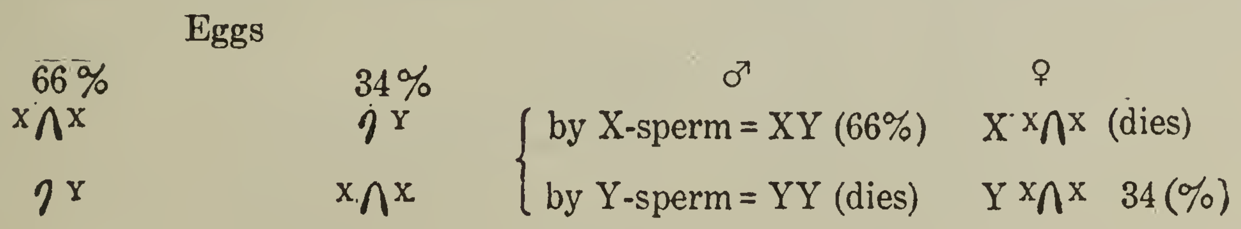


DIAGRAM 5

On this view there will be more Y-bearing eggs (after reduction) than attached-X eggs. When these are fertilized by the X-sperms, two classes of zygotes result in the equal numbers, *viz.,* males (XY) and three-X females. The former survive, the latter rarely come through. If the eggs are fertilized by the Y-sperms, two classes of zygotes are also formed, *viz.,* YY zygotes that die, and XXY females. The number of the latter will be determined by the number of eggs that are left (after reduction) with the attached-X chromosomes. Thus, while in the ordinary attached-X stock half of the zygotes fail to come through, the proportions here are changed; for, the excess of males and the deficiency in females will be invariably proportional to the unusual reduction process here assumed to occur. Whether the ratio of females to males be not very low (1 to 2) or exceptionally low (1 to 20) half of the zygotes fail to come through, and in the half that survives the ratio will depend on the extent to which the attached-X's are eliminated.

There is a theoretical possibility of distinguishing between the first hypothesis of the semi-lethal genes and the second of disproportionate elimination of the attached-X's. For example, on the first view 100 zygotes out of 200 eggs die irrespective of the ratio, *viz.,* the XXX and the YY individuals. On the second view, should only 5 females come through in each 200 eggs, then 145 zygotes (probably in the egg stage) are expected to die. If more females come through, the number of dead zygotes approximates that expected in the first view. Whether this difference could be detected may seem doubtful, both because some eggs usually fail to hatch in any cultures, and because the chance of getting a culture giving the excessively low female ratio here assumed would be very small.

If, however, this explanation could be established it would furnish a novel situation. If a change in the attachment of the X's occasionally occurs we should expect the ratio from a given female to be constant. It might seem possible to test this view then by placing a female under varying external conditions. If the ratio remains constant the effect is internal, if variable, the environment might be held responsible. Such tests as have been made (few in number) have been inconclusive because the limited number of offspring, which the attached-X stock usually gives, is insufficient to furnish conclusive ratios.

FURTHER RECORDS OF THE BROKEN-YELLOW STOCK

The broken-yellow stock, bred in small mass cultures during the years 1926–1927, gave average ratios of nearly two males to one female. Mass cultures of this stock, that gave the ratios shown in table 24, were tested.

TABLE 24

	yy ♀	y ♂
I	33	69
II	45	101
III	44	84
IV	33	79

Some of the virgin females were mated to wild-type males and gave the following ratios (table 25).

TABLE 25

	Gray ♀	Yellow ♂
I	30	112
II	40	103
	19	59
	85	117
	102	137
	44	92
	59	129
	45	125
III	60	84
IV	14	52
	11	59
	32	132

The results show positively that the yellow-bearing chromosomes were still detached in these stocks that were giving low-producing females and that when outbred the ratios were still as low as in the broken yellow stock.

That the low ratios are not due to pair matings in comparison to the parent YY-stock (I to IV, table 24) was demonstrated by making twenty-two pair matings from the same stock (two generations later than table 24). The results were as follows (table 26).

TABLE 26

	yy ♀	y ♂
from (a) (92 ♀ by 138 ♂)	69	60
from (b) (84 ♀ by 154 ♂)	43	78
	56	101
	42	71
	34	43
	46	67
from (c) (80 ♀ by 143 ♂)	59	76
	45	85
	67	77
	39	51
	50	80
	35	63
from (d) (54 ♀ by 69 ♂)	52	89
	53	69
	54	91
	42	70
	48	91
	59	79
from (e) (83 ♀ by 126 ♂)	56	70
	13	12
	55	84
	38	67

The ratios here are the same as in table 24 except one (69 ♀ : 60 ♂) which is practically 1 to 1.

As a sort of control, the same yellow males of the broken stock were mated to females (in pairs) of the attached-X, low-female-producing stock. The result is given in table 27.

TABLE 27

yy♀	84	3	15	3	13	2	6
y♂	84	7	86	28	48	8	22

With the exception of the first pair the female ratios are as low as in the last case. This means only that the males behaved in the combination in the same way as any other males.

There was the possibility that the X's might sometimes reunite in those selected lines that give the low females. This was tested by outbreeding to Bar males of low female stock. Had the X's reunited, the sons would have been like the father as to sex-linked characters; if they had remained apart the sons would have been like their mothers as to the same characters. The test gave the latter result, *viz.,* 69 het. Bar females : 61 yellow males, and 38 het. Bar females : 26 yellow males, showing that the X's had not reunited.

DAILY RATIOS FROM THE SAME CULTURES

If the fluctuations of the low female ratio from attached-X stock is connected with environmental conditions this might have become apparent if the same lot of flies is changed each day to a new culture bottle. This was tried with 18 females and as many males. At first the change was made every day, then every other day.

TABLE 28

	yy♀	B♂	XXX♀		yy♀	B♂	XXX♀
Mon.	30	226	2	Mon.	43	115	2
Tues.	42	69	....	Wed.	68	132	1
Wed.	50	205	....	Fri.	75	275	1
Thurs.	65	145	4	Mon.	32	90	.
Fri.	64	179	3	Wed.	48	99	.
Sat.	81	201	5	Fri.	15	54	.
				Sun.	41	86	1 N♀ notch wing

These ratios vary from less than 1 : 2 at one extreme, to 1 : 7 at the other extreme. These mass cultures are not satisfactory because different females may have laid eggs on different days. The test should be repeated with single pairs, and since this stock does not produce many flies, smaller bottles should be used to assure good cultural development. On the other hand, as the stock was practically homogeneous at this time, the variability in the reaction may be safely ascribed to environmental conditions.

EGG COUNTS

In order to find out where the mortality (if there be such) of the missing females occurs, "egg counts" were carried out. These counts were made for me by Mrs. Dobzhansky and I am greatly indebted to her for her skill and patience in carrying through such a laborious piece of work. Five such series are recorded in table 29. The mothers came from attached-X, yellow stock that was giving very low ratios. They were bred to wild type males, and eggs from several females, as laid, were counted and isolated by the usual procedure. The larvæ, pupæ, and adults were recorded for each set. The larvæ were reared on fermented banana and under very favorable conditions.

Table 29

	eggs	larvæ	per cent	pupæ	per cent	adults	per cent	♀	♂
A.......	50	36	72	25	69.4	13	52	7	6
B.......	127	91	71.7	62	68.1	46	74.2	14	32
C.......	120	87	72.5	50	57.5	37	54	18	19
D.......	228	155	68	90	58.1	75	83.3	34	41
I........	492	334	68	248	74.2	198	75.8	82	116
Total..	1017	703	62.12	476	67.7	369	77.5	155	214

The table shows that there was a mortality in the egg stage of about 25 per cent. This probably consists of the YY-individuals that die in the egg stage, as has been shown by Dr. Li to take place. He also found that another 25 per cent, consisting of the XXX flies, die later. In only two cases did the females fall significantly behind the males (14 : 32, 82 : 116) and only one of these was 50 per cent behind. In the other three cases the sexes were approximately equal.

The pupæ also ran considerably behind the larvæ but not more so in the lower ratio cultures. The mortality is not present to a greater degree in the two low ratio classes than in the others. Again the adults ran behind the pupæ but not more so in one case than another.

It is not evident from these results that the deficiency in females could be assigned to any particular stage. Moreover the unfortunate absence of any very low adult ratios in these counts makes it impossible to draw any conclusion from the evidence bearing on the point at issue.

A culture of wild-type males and females made at the same time to test the method showed scarcely any mortality as shown in table 30.

Table 30

	eggs	larvæ	per cent	pupæ	per cent	adults	per cent	♀	♂
+.......	174	172	98	151	87.8	146	96.7	74	72

If any conclusion can be drawn from this evidence it is that the conditions were so favorable that few or none of the attached-X females died.

Later, the experiment was repeated. The offspring of two generations were first recorded. The stock used had double-yellow females and normal (gray) males. The ratio of daughters to sons is given in table 31. More males than females were present, but not twice as many. Some of the daughters were next bred to wild stock males ("Oregon"). The offspring are recorded in table 32. The ratios are not different from those in the preceding case. Some of these (F_2) yellow daughters (attached-X) were then mated to yellow males of stock. This was done in order that the offspring would all be yellow and therefore more nearly alike in viability, or at least the yellow males here would be expected to be more nearly comparable as to viability with their double-yellow sisters than were the gray males in the preceding generations. The "egg counts" are given in table 33.

TABLE 31

	♀	♂	total
A	67	99	166
B	92	120	212
D	63	101	164
I	130	157	287
Total	352	477	829

TABLE 32

	♀	♂	total
A	77	111	188
B	69	109	178
D	79	84	163
I	176	183	359
Total	401	487	888

TABLE 33

	eggs	larvæ	per cent	pupæ	per cent	adults	per cent	adults	
								yy♀	y♂
A	214	161	75	107	66.5	107	100	51	56
B	228	176	77	118	67	118	100	64	54
D	81	58	71	36	62.1	36	100	15	21
I	332	241	73	167	69.3	167	100	77	90
Total	855	636	74.4	428	67.3	428	100	207	221

In none of the series was there a low female ratio. Hence this evidence can have no significant bearing on the occasions where such ratios are present. As in the earlier egg counts many eggs failed to hatch—about 25 per cent. There was also a loss before pupation, but all of those that pupated became adult flies. Taken at their face value, the absence of low female ratios in these experiments would appear to be due to the favorable conditions under which the larvæ lived—being transfered to fresh food after hatching and not crowded. If this is the explanation the usual low female ratio would then appear to be due to the failure of the attached-X female to survive under poor food conditions; for, the result can not be ascribed to "crowding" as such, because the attached-X's are so little productive or so slow in reproduction that no crowding occurs. In the absence of many larvæ, however, the food is often not worked over and generally is poor. It is to this element that the deaths must be ascribed if the culture conditions are responsible for the low ratios. This means that these females are less resistant than their brothers that do not have the attached-X's.

SUMMARY

Stocks in which the two X-chromosomes have become attached to each other give equal numbers of females and males, as a rule. Half the females (XXX) die in most cases, and half the males (YY) also die. At times females have appeared in these stocks that produce many more sons than daughters and this peculiarity is transmitted along the female (XX) line. When these females are mated to males of other stocks the unusual ratios persist. When the males of the low-producing female line are out-mated to ordinary females they never transmit the peculiarity of their stock to other lines.

The usual tests to determine whether genes in the chromosomes other than the X's carry specific factors for the unusual ratio gave negative results. This applies to chromosomes II, III and IV.

Occasionally the attached-X's break apart, and if, as in the present case, each X carries a gene for yellow, a yellow son is produced. It is possible by utilizing such a yellow male to produce a new stock (without allowing crossing over between the yellow-X and the X introduced into F_1) with the original yellow chromosome (now "detached") in the females. In this way it was hoped to determine whether the unusual ratios are due to genes carried in the attached X's, or else to some peculiarity in the attachment. The stock of detached X's gave at first some normal and some unusual ratios. By selecting from the latter a new stock was produced that gave low female ratios. Numerous outcrosses showed positively that the X's were still detached.

Even if the X's of the low-producing stock carry recessive lethals, it does not follow necessarily that, when they become separated, each will carry the lethal, for, if the lethal were at or near the junction it might be absent in one such chromosome. In fact the survival of the resulting male might in some cases depend on the absence of the lethal; but if on the other hand it is only semi-lethal some of the resulting males might come through as do some of the females.

That simple attachment of the X's does not in itself produce low female ratios may seem evident because the ordinary stock of attached-X's does not give these ratios. But occasional changes in this stock when a low-producing female appears, might be explained as due to some shifting in the method of their attachment that leads more frequently to the elimination of the attached pair than to the Y-chromosome. A cytological comparison of the two kinds of stock furnished no positive evidence in favor of this view, but such evidence has only a negative value.

Egg counts were made to detect, if possible, the time of occurrence of a significant mortality. None of the tests gave, however, sufficiently low ratios to furnish convincing evidence. It is suggested that the more favorable conditions of the larvæ may account for the absence of the low ratios in these counts.

The evidence at hand points to a semi-lethal factor or factors carried in the attached-X's. The environment acts as a differential on females of this genetic composition.

In so far as the discussion applies to the presence of a semi-lethal gene or genes the question of the nature of the genes is left open. It is not intended to suggest that the gene is a "point mutation" to the exclusion of deficiences, translocations, or inversions.

When females with two separate (detached) chromosomes derived from low-female-producing stock—the detached stock also giving low-female ratios—are outbred to "normal" males they give low-female ratios. The result proves either that the low-producing (semi-lethal) gene is dominant, or else that, being recessive, the two genes have already produced their effect on the egg before maturation. The former hypothesis is disproven by the first generation of offspring from detached X's, which do not give low ratios, and also by out-mating males from detached X's of low-female-producing stock. These should give low ratios if the X brought in by the male is dominant, which they do not give. The other conclusion, namely, that the semi-lethal effect is produced on the egg before maturation is consistent with the results stated above. The crucial test would come in the next F_2 generation following the F_1 of table 25. These females if outbred should not then give low ratios, even if a detached-X male of low-producing stock were used.

V

VARIABILITY OF EYELESS

By T. H. Morgan

VARIABILITY OF EYELESS

BY T. H. MORGAN

SELECTION OF EYELESS

Eyeless is a mutant whose gene is in the small fourth chromosome. Several stocks are kept that arose independently. Either they have allelomorphic genes for eyeless, or their differences may be due to modifying factors. One, the original eyeless, was found by Miss M. A. Hogue in 1914; the other, eyeless 2, was found independently by Dr. Nonidez and the writer, in the spring of 1919 in the same stock. Another, eyeless 4, was found by Dr. Li (1925). In all the original stocks the eyes were very variable as indicated in some of our earlier figures of eyeless (*Bibliographia Genetica II*). In some individuals both eyes are present and nearly full size; in others both may be small, equal or unequal in size; in some individuals one eye is present (large or small), the other absent. In still others both eyes are absent. The three ocelli are generally present, although often—especially where one or both compound-eyes are absent—one or more may be displaced, or extra ocelli may be present.

The stocks are now kept with little selection, and remain at about what may be called the upper level, *i. e.*, flies having both eyes, one eye, or no eyes. When both eyes are present they are, as a rule, smaller than normal, but often approach the normal so nearly that they cannot be separated and even overlap normal. The eyes are often very unequal on the two sides.

METHOD OF SELECTION

In general, two methods of selection were followed. In each generation pair-matings were made and the most extreme individuals were selected as the parents of the next generation. Such a procedure is best if the results of selection are to be followed carefully in each generation. On the other hand, those flies that are without compound-eyes are blind, and pair matings are not so successful; for, the individuals may not mate for so long a time that the food becomes mouldy and the flies themselves get entangled in the mould. But if in each generation, small mass cultures of 6 to 12 flies are used, choosing as far as possible only virgin females, the chance that the culture will succeed is far greater. It is also a time-saving procedure. The determination as to what has taken place in the stock can then be attempted after the selection has gone so far that no further changes are apparent.

In order to find out when selection had ceased to bring about further modification, some method had to be devised to give a measure of each successive generation. The difficulty was to find some convenient way to measure the eyes. No doubt, measurements of the eyes would give the most accurate data, but this is too laborious, and technically too difficult, as also would be an attempt to count the ommatidia. I resorted to the simpler method of recording whether each fly had no eyes at all, or one eye, or both eyes, regardless as to whether the eye, when present, was large or small.

It soon became apparent that any accurate measure was out of the question, since each culture undergoes a change from day to day. As the culture becomes older, the number of flies with one eye or with both eyes steadily increases in number. In extreme cases none of the flies in a given culture had eyes at the first count, while on the tenth day all the flies might have two eyes, or one at least. Since the cultures do not all go at the same rate, it is difficult to institute comparisons between them except in a very general way.

The change in the character of the offspring is not due to an age difference in the parent; for, if the old flies are taken out after 9 or 10 days, and put into fresh bottles, their first offspring are the same kind in the second bottle as those that had been produced in the first bottle.

It might be supposed that the eyeless flies hatched first and those with eyes later, but that this is not the explanation of the change was shown by moving the old flies from day to day to new bottles when all the offspring that hatched were like the first counts of a bottle.

CHARACTER OF EYELESS

When the eye is entirely absent the area that it would usually occupy is covered by a smooth cuticle. Since this area does not bulge out as does the normal eye, the head has, from side to side, a laterally contracted appearance as seen from above. The absence of the outer optic ganglia may also contribute to this reduction in size. The condition of the brain and ganglia has been described by Mrs. Richards.

When a small eye is present, it sometimes occupies the center of the optic area, and is surrounded by a cuticular zone representing the missing part, but it may lie in an eccentric position. The small eye is as a rule circular or slightly oval in outline, sharply bounded, and composed of ommatidia approximately normal in size, but fewer in number than normal. In extreme cases, the eye has only two or three ommatidia, and sometimes only a splash of red pigment is present.

ANTENNÆ IN EYE

The development of an antenna in the eyeless region is of frequent occurrence. Some duplicated antennæ have been figured in *Bibliographia Genetica,* 1925, Vol. II, 72. The antenna, if in the eye region, has nearly always the same orientation as that of the normal antenna of the same side. In other words, it is not a mirror image or duplication of the normal antenna of the same side, but an organ, in series, equivalent structurally to a normal antenna. On the other hand, the antennæ may appear in duplicate and be mirror figures. These are always near together and arise as a rule from a single basal joint. It is not without interest to note that the antenna that replaces the eye is usually quite perfect in structure and size. It may be recalled that in certain crabs, Herbst has shown that an antenna develops if the eye and optic ganglion are removed. The physiological significance of these relations is obscure.

According to recent work by Chen on the imaginal disc of the eyeless *Drosophila* the antennal part of the cephalic imaginal discs is present and of normal size even when the eye part of the common disc is almost completely absent. It may be that the absence of the eye portion of the disc has a close relation to the doubling of the antennal portion, but just what this means is not apparent. It might mean more space for the development of the antennal part which causes it to double, or some difference in the shape of the antennal part leads to duplication. On the other hand, it may equally well be supposed that the extra antennæ come from the rudiment of the optic portion of the disc when it is present.

TROPISMS OF EYELESS

The adult normal-eyed *Drosophila* is strongly positive in its light reaction, both when it crawls and when it flies. At rest, however, it is almost indifferent and may even stand turned away from light. The flies without compound eyes show no reaction to light, although the ocelli are present. It follows that the ocelli of the flies have no significance in normal orientation, and also that the light does not act directly on the brain, or at least not on that of the eyeless flies. It would be hazardous to extend this conclusion to other insects, for it is possible, though unlikely, that the ocelli of eyeless are affected by the other conditions present in the flies. When the flies have small eyes on both sides they show a positive reaction, but whether it is as quick or as accurate as that of normal-eyed flies has not been made out.

When the flies have a large eye, or even a small eye, on one side only, they show circus movements as they crawl, whether illuminated from one side or continuously from above. If illuminated from one side, the fly, if starting to the light turns to the eye-side and, as it does so, tends to place the eye in the shadow of its own head. This, however, stops the turning unless enough reflected light is present to cause a continuation of the circling until the eye emerges on the opposite side into the light again, etc. When illuminated from above the circling is due to a light continually impinging only on one side—there is no movement here towards the light, but one towards the side that is affected by the light through the eye.

When a fly has one large and one small eye, it often shows circus movement towards the large eye.

PROGRESS OF SELECTION FOR NO EYES

The experiment began in January 1921, at Palo Alto, California, by selecting a few flies from stock that had small eyes. In the second generation the selection was reversed, *i. e.,* selection of big eyed flies took place. The following selections were "downward" again. Definite records of the condition of the eyes began to be gathered only in the seventh generation. Some of the records at this time, as seen in the Appendix, are as extreme as those of today (1926), but others are by no means so good as at a later date. This means that the genetic possibilities for extreme eyeless were already present in the seventh generation, and probably, therefore, in the stock at the start. That the genetic factors that assist in producing a larger number of flies without eyes in the early counts had not been at the time segregated, is shown by selecting as parents the flies that had the two largest eyes. The response was immediate, giving a greatly increased number of flies with both eyes, and relatively fewer with one or with no eyes.

As stated above, the first requisite for a study of the effects of selection on the eyeless character was to find some approximate objective measure of the condition of the eyes. The flies were sorted out into three classes, *viz.*: (1) without eyes (2) with one eye (3) with both eyes. Experience showed that when there were many flies with no eyes in a culture, those with only one eye were few, and those with two eyes scarcely present at all. Conversely, if many flies have both eyes, there are many with one eye, and very few with no eyes.

The classification, unfortunately, does not express another relation, namely, that when many flies in a culture are without eyes, those with one or with two eyes have as a rule much smaller eyes than the single-eyed and two-eyed individuals of the original stock. In order to show this relation a rough estimate of size of the eyes was taken in 1922. The data are given in tables 1, 2, 3. It is evident that there is a high correlation between the total absence of one eye and the size of the other eye when present.

TABLE 1—*Unselected Eyeless Stock*

L	¾	¾	½	¾	¼	¾	⅛	¾	0	½	½	¼	½	0	⅛	¼	¼	⅛	¼	0	⅛	0
R	¾	½	¾	¼	¾	⅛	¾	0	¾	½	¼	½	0	½	½	¼	⅛	¼	0	¼	⅛	0
No.	123	21	34	9	6	2	1	4	3	15	3	6	2	4	2	5	2	1	1	1	1	1

L>R L=R L<R
44 145 58

TABLE 1A—*Summary of Table 1*

¾		½		¼		⅛		0	
R	L	R	L	R	L	R	L	R	L
167	159	48	54	19	20	5	5	8	9

It has recently been shown by Chen that the imaginal eye-disc of the completely eyeless-fly is almost totally absent. Furthermore the larvæ of the late hatching "eyeless" flies in which the eyes begin to appear, show corresponding changes in the amount of development of the eye disc, even at an early stage. Presumably when a difference appears on the two sides of the same fly, this difference also is present in the discs although this is not reported by Chen.

In order to find out something about the difference in size of the eyes in the same fly, a classification of the condition of the eyes in several stocks was made. In each fly, the right and the left eye was classified as full eye, ¾ eye, ½ eye, ¼ eye, ⅛ eye, and no eye. After these data had been collected, the classes were sorted out according to size. When both eyes were present each was put into its own class; when only one eye was present (the other absent) it was of course recorded once on its proper side. The condition of the control eyeless stock at this time ("Woods Hole") is shown in table 1. Nearly all the flies have two eyes that are ¾ or ½ full size.

A summary of table 1 is given in table 1*a,* in which all the eyes that are ¾ or ½, etc., are classified according as to whether each is on the right or the left side. There is no observable tendency for the eyes to be larger on one side (right) than on the other (left). Both eyes were recorded as of the same size in 145 flies and unequal in 102 flies.

TABLE 2—*Select Up Eyeless 20th Generation*

L R	¾ ¾	¾ ½	½ ¾	¾ ¼	¼ ¾	¾ 1	1 ¾	½ ½	½ ¼	1 ½	1 ¼	1 1	⅜ ⅛	¾ ⅛
No.	91	3	7	1	4	11	10	2	1	2	1	54	1	1

L>R	L=R	L<R
20	147	22

TABLE 2A—*Summary of Table 2*

1		¾		½		¼		⅛		⅜	
R	L	R	L	R	L	R	L	R	L	R	L
65	67	112	107	7	10	3	4	2	0	0	1

One eyeless stock had been selected through 20 generations for large eyes ("select up"). Its condition is recorded in table 2. All the flies had two eyes and nearly all were approximately full size or ¾ size. Again,

TABLE 3—*Select Down Eyeless 23d and 24th Generations*

L R	¾ ¾	¾ ¼	¾ 0	0 ¾	½ ½	½ ¼	¼ ½	½ 0	0 ½	⅛ ½	¼ ¼	¼ 0	0 ¼	½ ⅛	⅛ 0	¼ ½	0 ⅛
No.	1	1	3	6	7	5	1	18	20	1	4	15	26	1	3	1	4

L>R	L=R	L<R
46	12	59

TABLE 3A—*Summary of Table 3*

1		¾		½		¼		⅛		0	
R	L	R	L	R	L	R	L	R	L	R	L
0	0	7	5	30	31	36	21	5	4	39	56

TABLE 4

	Both	One	None
Select down (19th generation)	0	7	51
	0	7	22
	0	0	4
	9	16	11
	7	21	9
	24	23	3
Select down (19th generation)	0	9	68
	1	3	17
	29	25	7
Select down (18th generation)	0	6	60
	0	3	10
	0	4	4
	1	6	12
	6	7	7
	24	18	8
	10	3	3
	5	9	9

TABLE 5

	Both	One	None
Select down (21st generation)	0	1	20
	0	2	25
	0	0	29
	2	1	42
	5	3	33
	0	5	6
Select down (20th generation)	0	12	71
	1	13	21
	0	2	13
	2	9	36
	5	7	10
	18	8	6
	1	12	25
Select down (21st generation)	0	1	7
	0	0	16
	0	2	47
	0	2	21
	0	4	7
	2	0	8
	2	3	29
	0	9	28
Select down (21st generation)	0	0	1
	0	0	2
	0	2	8
	0	2	22
	2	11	53
	2	6	9
	0	7	11
	0	1	3

TABLE 6

	Date	Both	One	None
	..	0	3	61
	15	0	7	46
	16	2	8	18
Food added..........	17	3	6	9
	18	1	0	7
	19	1	5	3
(19th generation)	20	4	5	6
	21	5	8	1
	22	25	11	4
	23	7	14	15
	25	13	18	60
	26	0	11	35
	27	2	4	22
	21	0	1	29
	22	0	6	42
(19th generation)	24	2	5	53
	25	0	4	20
	26	4	10	3
Became dry.........	2	6	19	9
	26	0	0	1
	26	0	2	15
Kept feeding.........	27	1	4	38
(20th generation)	2	3	9	135
	4	2	24	53
	6	3	10	26
	7	0	5	32
Food added..........	23	0	0	5
	24	0	0	13
	25	0	0	12
(20th generation)	27	0	1	12
	5	4	31	105
	7	8	24	29
	14	0	0	30
	14	0	7	11
	15	0	0	20
	16	2	4	22
	17	0	0	11
	18	1	5	11
Food added several	19	0	1	3
times.............	20	1	1	0
(21st generation)	21	2	2	1
	22	7	9	3
	23	18	15	9
	26	17	32	71
	27	2	2	10
	2	0	2	3
	4	0	0	2
	12	0	13	74
Food added..........	16	11	26	57
	17	3	0	1
(21st generation)	18	3	3	3
	19	0	0	1
	23	4	4	2
	28	2	1	15

there is no asymmetry observable (table 2*a*). Both eyes were recorded as the same in size in 147 cases, and as different in only 42.

Another stock derived from the same original stock had been "selected down" for 23 and 24 generations. Its condition at the time is recorded in table 3. Here most of the flies have eyes only ½ and ¼ the full size. There is no asymmetrical distribution observable on the two sides (table 3*a*). The eyes on the two sides of the same fly were recorded as equal in only 24 cases, and as different in 209 cases. The difference is so large that it cannot be regarded as due to greater ease of observing the differences that exist, but indicates rather that there is a much greater variability in the size of the smaller eyes. A very considerable number of these flies lacks one eye completely. This occurred 78 times on the right side and 112 times on the left side. It seems doubtful if this difference is indicative of an asymmetrical relation.

Another consideration shows that it would not be worth while to spend much time making accurate measurements of the eyes. As the culture gets older from day to day the character of the population generally changes, depending on the kind of alterations that take place in the food. A characteristic change is illustrated in the three counts recorded in table 4, each from a single bottle, at intervals of about two days. These counts show that while at first nearly all the flies are without eyes, there is a shift until nearly all have both or one eye at least. On the other hand, if the fermentation goes well and the bottle remains moist there may be no changing-over during the first seven or eight days, as the four counts in table 5 show. By adding new food (fermenting bananas) every few days the change may be to a large extent prevented as shown by the six records in table 6. The days on which the counts were made are given in the first column.

It has been pointed out that this shift is not due to the increase in age of the parents, for if transferred to a new bottle their offspring repeat the story. The result is due most probably either to the amount of food or to some other change in the environment.

It is not a little curious to find that the best fed and biggest flies are those less likely to develop eyes, while the late hatched small starved flies have large eyes.

PROGRESS OF SELECTION "UPWARD" FOR FULL EYE

A few flies whose eyes were not quite full were picked out from stock and bred together. From their offspring and in subsequent generations, flies with eyes as nearly full as possible were picked out to start each new generation. Almost at once the effect of selection was evident. In the seventh and eighth generations, after selecting "upwards" a census was made with the results given in table 7.

CROSSES BETWEEN STOCKS

Crosses were made between the up and down (+ and −) selected stocks in the seventh, eighth and ninth generations. In each case the flies were taken from the most extreme bottles of their kind. The results are given in table 8.

It is quite evident that the more nearly full-eyed (plus) stock is, in a sense, the dominant. It follows that for testing the F_1 flies in order to find out if the difference in the two stocks is due to modifying factors, the "select down" eyeless (as the more recessive stock) should give more valuable results.

TABLE 7

	Both	One side fleck of eye	One side eyeless
Select up 7	247	0	2
	120	0	0
	74	0	0
	157	0	0
Select up 8	278	2	2
	127	3	0
	154	2	0
	119	60	12
	51	0	0
	417	1	0
Select up 10	47	0	0
	66	0	0
Select up 11	160	0	0
	173	1	0

TABLE 8

	Both	One	None
Select down 7 ♀ by select up 7 ♂	24	9(Intermediate)	
Select down 7 ♀ by select up 7 ♂	84	(All big to intermediate)	
Select down 8 ♀ by select up 7 ♂	26	14	7
Select down 8 ♀ by select up 9 ♂	138	19	3
Select down 8 by select up 9 ♀	15	2	0
Select down 8 by select up 9	46	2	0
Select down 9 by select up 9	19	16	2
Select down 9 by select up 9	264	46	9
Select down 9 by select up 9	27	7	0
Select down 9 by select up 9	28	0	0

The difference in the plus and minus stocks present in the 13th to 17th generations of selection is to some extent brought out by the following experiments in which flies from each stock were first crossed to Curly Hairless stock and the F_1 back crossed to either the plus or minus selected stock. For example, select-down "eyeless" flies (14th and 17th generation) were mated to Curly Hairless. The F_1 Curly Hairless males were bred to select-down flies. The results are recorded in table 9.

In another case select-up flies (14th, 16th, and 17th generations) were bred to Curly Hairless, and the Curly Hairless F_1 males were back-crossed to select-down (16th generation). The results are shown in table 10.

The difference in the two cases is not conspicuous. On the assumption that the Curly Hairless stock brings in modifiers for eyeless, especially those in the plus direction, their redistribution in the second generation would tend to cover up the recessives in that generation. On the other hand, when the select-up flies (13th and 14th generations) were bred to Curly Hairless and the F_1 males were back-crossed to select-up flies (15th generation) the results are somewhat different from those of table 10 as shown in table 11.

Table 9

Normal eyes	Eyeless		
	Both eyes	One eye	None
(1) 154	154	57	2
240	25	7	0
(2) 230	39	1	0
285	11	0	0
160	20	1	0
250	25	0	0

Table 10

Normal eyes	Eyeless		
	Both	One	None
5	2	0	0
44	11	2	0
64	24	9	0
60	17	15	2
126	24	8	2
17	10	7	1

Table 11

Normal eyes	Eyeless		
	Both	One	None
114	4	0	0
169	29	0	0
41	8	0	0
31	5	0	0
41	3	0	0
15	2	0	0
176	41	3	0

Here all except three of the F_2 eyeless flies had both eyes present. The result may be interpreted to mean that the Curly Hairless brought in plus modifiers which were similar to or the same as those in the select-up stock. A census of the select-up stock in the 18th generation gave 350 flies with both eyes, one fly with one eye and none with no eyes. The outcrossing had then virtually no effect on the condition of the eyes in the select-up eyeless stock. By implication, the select-down stock owes its difference to the accumulation of recessive factors that tend to obliterate the eyes.

LOCATION OF THE MODIFYING FACTORS FOR EXTREME EYELESS

The usual method for locating factors by the use of dominants in the second and third chromosomes was applied to eyeless. The first tests were made when the selection had gone only as far as the 7th and 8th generation of selection. The results were not wholly satisfactory, indicating perhaps that the stock was not yet homozygous, and the experiments were repeated later. Nevertheless a few examples may be given that will serve as an introduction to the difficulties that this case presents.

An eyeless female of the "select down" stock in the seventh generation was bred to a Curly male—a dominant second chromosome mutant. All the offspring had full-sized eyes. The F_1 males that were Curly were back-crossed to females of the select down stock in the seventh or eighth generation. The F_2 (back-cross) offspring are given in table 12.

TABLE 12

	N	Cy	ey	Cy ey	Both	One	None
F_1 Cy ♂ (Cy by down 7) by down 7 ♀..	9	8	4	5	2	2	5
F_1 Cy ♂ (Cy by down 7) by down 8 ♀..	26	20	25	20	16	18	9
F_1 Cy ♂ (Cy by down 7) by down 8 ♀..	13	20	0	9	2	6	..
	48	48	29	34			

The flies were first separated into the eyeless and not-eyeless groups, and then each group subdivided into Curly and not-Curly (*i. e.*, normal wings = N). In addition, in each cross, *samples* of the eyeless flies were classified according to whether both eyes, one eye, and no eyes were present.

If the eyeless flies were as viable as the normals, equal numbers of these two classes are expected. In fact, however, there were 96 normals and 63 eyeless, but since it is known that some of the eyeless flies have full eyes (or eyes that appear to be full size) the excess of normals may be due, in part, to their inclusion in this group. On the supposition that the presence of modifying factors increases the percentage of flies without any eyes, it is to be expected that in those F_2 classes of eyeless in which the original chromosome complex has been recovered there will be a higher percentage of flies with no eyes than in those classes with a new combination of chromosomes, which here is the class with the Curly chromosome. Unfortunately, this subdivision was not made at this time in such a way that this can be shown, but it was made in later experiments. There is, however, another criterion that may be applied which is not so good. If it is assumed that the modifier, that makes for more flies with no eyes, also increases the chance that in the F_2 generation there will be a higher percentage of eyeless,—*i. e.,* fewer of the genotype, "eyeless" flies having full eyes, then when the original complex of chromosomes is recovered (as in the not-Curly, eyeless class) there will be more eyeless flies than in the class that contains the foreign chromosome, *viz.,* Curly. As a matter of fact, no marked difference is observed and such as it is (the difference present is probably not significant), it is in the opposite direction.

The preceding discussion rests on the assumption that the modifier is recessive. If it is dominant no such differences will be expected in the F_2 generation.

These results, as far as they go, furnish at least no evidence that there are recessive modifying factors in the second chromosome. A similar test was made for the third chromosome using the dominant factor, Hairless. One experiment gave aberrant results and is discarded. The other is given in table 13.

TABLE 13

	N	H	ey	ey H	Both	One	None
F_1 S H ♂ (H by select down 7) by down 7.....	25	25	14	16	12	7	11

The results are similar to those for Curly and do not furnish evidence of a recessive modifier in the third chromosome.

Since these two experiments were made with different stocks, namely Curly and Hairless, they are open to the objection that each stock may have introduced different modifiers that affect the result. In order to avoid this, the experiment was repeated using both dominants at the same time. Curly Hairless males were bred to selected-down eyeless females in the seventh and eighth generation, and the F_1 Curly Hairless males were bred to selected-down eyeless females of the ninth and tenth generation. The results are shown in table 14.

TABLE 14

	H	ey H	Cy H	ey Cy H	N	Cy	ey	ey Cy	Eyeless		
									Both	One	None
F_1 CH ♂ (Cy H by down 7) by down 9 ♀.............	10	3	12	7	10	18	1	4	4	9	8
F_1 CH ♂ (Cy H by down 7) by down 10 ♀............	17	4	4	6	10	20	6	8	9	9	3
	5	0	3	2	0	1	1	2	6	0	0
F_1 CH ♂ (Cy H by down 8) by down 10 ♂............	12	2	22	11	10	12	1	6	9	9	3
	44	9	41	26	30	51	9	20	28	27	14

Only about one-third of the flies are eyeless (166:64). Comparing class by class the eyeless with the not eyeless, there is no consistent evidence showing that the percentage of eyeless is higher when the original second and third chromosomes are present than when one or the other is derived from a foreign source, namely, the Curly and Dichæte chromosomes. This is apparent in the following tabulation.

When Hairless is present.................... = 44 full 9 eyeless
When Curly is present...................... = 51 full 20 eyeless
When Hairless and Curly are present.......... = 41 full 26 eyeless
When neither is present..................... = 26 full 9 eyeless

Owing to the environmental influence on the condition of the eye, it seemed very questionable whether the foregoing differences have any genetic significance, especially since they do not appear consistent amongst themselves. Therefore, an experiment was carried out on a larger scale with later generations (15th and 16th) when the homozygous conditions of the stocks was better assured. It did not seem necessary to classify the flies with full eye into their respective classes (as was done above) and the separation was made only of the flies with eyes smaller than full. In this material, one might expect to get evidence of the modifying factors, if present, that make more flies without any eyes. The results are given in table 15.

The numbers of eyeless flies obtained would seem to be sufficiently large to show whether the second and third chromosomes carry the modifier in

TABLE 15

Bottle	Total	Full eye	Both eyes				One eye				No eyes			
			N	Cy	H	Cy H	N	Cy	H	Cy H	N	Cy	H	Cy H
O	37	22	2	0	1	1	0	1	1	1	1	3	1	3
ZB	40	25	6	3	0	1	3	1	1	0	0	0	0	0
AC	66	37	1	0	1	0	2	6	2	3	4	6	3	1
XX	76	39	1	0	6	3	0	8	0	6	7	6	0	0
AD	84	53	0	0	1	2	0	4	0	3	8	8	3	2
AE	91	54	0	1	5	1	1	2	2	5	1	14	0	5
PP	97	81	6	1	0	0	5	0	0	0	4	0	0	0
UU	98	81	8	2	0	0	3	2	0	0	1	0	0	1
E	128	68	0	0	3	3	1	6	5	6	10	9	6	11
ZZ	130	89	3	6	2	4	4	1	3	2	5	4	3	4
AB	133	32	6	6	2	3	2	12	10	13	13	13	10	11
QQ	145	69	0	2	4	6	7	6	4	8	10	11	3	15
WW	163	80	5	8	7	2	1	7	5	9	8	19	4	8
MM	193	138	4	2	2	1	2	4	4	6	14	7	3	6
BB	208	150	5	6	2	8	5	7	1	4	7	10	1	2
RR	240	139	0	6	11	10	10	7	11	5	11	8	6	16
LL	268	181	1	4	2	9	3	5	9	13	9	14	11	7
GG	297	178	0	6	11	11	6	11	6	12	22	18	6	10
YY	299	184	3	1	1	7	1	13	4	11	14	17	19	28
NN	319	309	7	2	1	0	0	0	0	0	0	0	0	0
OO	395	199	25	35	22	15	12	16	16	14	9	3	18	11
AA	396	327	1	2	4	6	7	5	6	6	18	6	2	6
HH	420	277	2	18	10	14	9	13	6	11	13	20	7	20
FF	428	350	12	6	5	2	17	5	3	5	6	5	8	4
SS	504	336	3	3	9	13	7	20	6	16	21	19	25	26
		3794	116	121	113	124	115	166	106	163	216	214	139	203

question. Taken as a whole, the condition of the eyeless flies is somewhere between that of the original stock and that of the select-down stock. For example, these F_2 eyeless flies if classified into three groups: with both eyes; one eye; no eye; respectively, give

Both eyes 474; One eye 550; No eye 772.

In comparison with the select-down stock of this generation, there are noticeably more flies with both eyes present. This disposes of the possibility that the gene for eyeless had been changed as a result of the selection.

Within the class, "no eyes," the class (216) with the original grouping of second and third chromosomes (= N in table 15) contained no more flies than the other classes, except the class (139) containing the third chromosome (H), but that this result is not significant is shown by the Cy H class which is not significantly lower than the N class, etc.

A similar analysis of the one-eye group leads to the same conclusion. Since there is little or no crossing over in the fourth chromosome—the one containing the eyeless gene—the redistribution of modifying factors for eyeless cannot be referred to this source.

There remains only the X-chromosome. That the modifiers are not carried in the X-chromosome seemed determined by the way the experiment was carried out. The F_1 male had received his X-chromosome from his eyeless mother of the selected-down stock. He was crossed to eyeless females of the same stock, hence all the second generation (back-crossed) flies had the X-chromosome of the selected-down stock.

The conclusion may seem to follow that the recessive modifiers sought for are not present in any of the chromosomes. It is true the Y-chromosome has not been considered. This came from the Curly Hairless stock, and is present in the F_1 male, and transmitted by him to his sons. If the effect were due to this chromosome, the F_2 sons should be more numerous in the no-eye group than the daughters. No such effect was observed. Moreover, if the effect were due to the Y-chromosome the male and female of the original select-down stock should be consistently different, but this is not the case.

The only conclusion that can be drawn is that the experiment was not made in such a way as to reveal the location of the factors present. This may mean no more than that environmental factors swamped the differences due to the genetic factors.

If the extreme eyeless condition (no eyes) is affected by specific *dominant* genes in either the second or third (or in both) chromosomes, these effects would not be brought out by the preceding tests, because the chromosomes containing them would be distributed at random to the F_2 Curly and Hairless eyeless flies. If the double dominant, assuming it not to be lethal, gives a more pronounced minus effect than a single dominant, the expectation would be the same as for recessive factors. Since the not-Curly not-Hairless eyeless flies were not distinctly different from the others, the experiments indicate that no such decided differences were produced by double dominants in comparison with single dominants. Moreover, crosses between select-up and select-down would be expected to show dominance in F_1, but the result was the opposite, namely, the select-up type was the more dominant.

It became necessary to show that some of these F_2 flies could be again changed over to give the original distribution of the characters. This was done. As shown in table 16, for F_3, F_4, and F_5, the extracted eyeless (from Cy H) were soon brought back to the extreme "eyeless" condition.

Table 16—*Extracted eyeless (out of Cy H)*

	Both	One	None
F_3	0	7	60
F_3	15	46	159
F_4	0	4	46
	0	3	44
	0	2	12
	0	4	22
	3	9	26
	9	9	23
F_4	0	1	50
	0	1	47
	4	13	23
	5	6	12
F_4	0	1	19
	0	2	40
	1	8	38
	1	9	13
	2	0	7
F_4	0	0	5
	0	4	64
	0	5	34
	2	3	23
F_5	0	2	9
	1	8	25
F_5	1	12	63
	1	7	57
F_5	0	2	65
	0	7	62

TEST OF THE SELECT-UP STOCK

Experiments had shown that the ordinary stock responded quickly to selection in a plus direction. As a result, nearly all of the eyeless flies had two eyes present and these averaged larger than the two-eyed flies of the minus selected stock. Presumably, there were genetic factors present at first that modified the eyeless condition in a plus direction. In the first two experiments, the cross was made with Curly; in the second three, with Hairless. The results are given in tables 17 and 18.

There is no evidence from these results to indicate that recessive modifiers are present in either of the chromosomes tested, but perhaps no answer is expected since the action of the introduced dominant modifying genes would be to remove more "eyeless" flies into the full-eyed classes. There is no more obvious increase in the full-eyed (Normal) than in the full-eyed Hairless class.

In another experiment, the selected up eyeless flies (up^9) were crossed to Curly Hairless, and the F_1 male back-crossed to up^9 (table 19). The fact that in F_2 the flies with the original complex (N and ey) were more nearly in a 1 : 1 ratio than were the other classes, may or may not be significant. The numbers are too small to be decisive.

TABLE 17

	N	Cy	ey	ey Cy	Both	One	None
F_1 Cy ♂ (Cy by up 7) by up 7........	34	26	23	20	19	2	0
F_1 Cy ♂ (Cy by up 7) by up 7........	41	44	33	27	142	3	0

TABLE 18

	N	H	ey	ey H	Both	One	None
F_1 H♂ (H by up 6) by up 7	48	45	43	31	38	2	0
F_1 H♂ (H by up 6) by up 7	17	33	10	20	28	2	0
F_1 H♂ (H by up 8) by up 8	75	111	39	17	31	1	0

TABLE 19

	H	ey H	Cy H	ey Cy H	N	ey	Cy	ey Cy	Both	One	None
F_1 Cy H♂ (Cy H by up^9) by up^9.	10	2	13	6	2	1	5	6	15	1	0
F_1 Cy H♂ (Cy H by up^9) by up^9.	26	5	30	4	14	16	21	8	33	0	0

Finally, in one experiment "select-down" flies were bred to the double dominant Curly Hairless (CyH) and the F_1 males bred to "select up." The results (table 20) do not show any irregularities in the different classes (Cy and H, etc.). None of the flies lacked both eyes, demonstrating the dominance of factors in the select-up stock, or that the CyH stock itself carried plus modifiers.

TABLE 20

	H	ey H	Cy H	ey Cy H	N	Cy	ey	ey Cy	Both	One	None
F_1 Cy H♂ (Cy H by down^7) by up^8..........	15	2	35	0	23	9	11	3	16	0	0
	15	5	23	6	23	32	13	6	24	5	0
	15	10	33	8	24	11	10	13	42	2	0
F_1 Cy H♂ (Cy H by down^7) by up^9..........	12	16	33	21	12	50	5	12	42	11	0
	22	20	66	13	20	53	8	23	54	12	..
	11	9	20	6	8	18	5	9	..	..	..
	15	12	24	2	19	17	15	13	..	..	..
F_1 Cy H♂ (Cy H by down^8) by up^8..........	33	11	50	11	35	38	19	12	42	11	0

SELECTION EXPERIMENTS

One of the objects of continuing the selection experiment in the minus direction, *i. e.*, by selecting flies with no eyes, was to discover whether a stock entirely without eyes could be produced, and whether after many generations of selection, reversal of the selection would bring about any change. In regard to this first point it may be stated that after 60 generations of inbreeding the eyeless flies continued to produce some flies with eyes which were, as a rule, smaller than the full eye. Late hatching flies continued, as at first, to have eyes approaching more nearly the normal in size.. This means that if the stock has become practically homozygous, either the gene for "eyeless" gives, as a phenotype, flies with small eyes as well as flies with no eyes and cannot be changed by selection, or else it means that the stock has become homozygous for genes that reduce the size of the eye which genes were probably present from the beginning. Here, as in all such experiments, it is difficult to discriminate between these two possibilities unless very elaborate linkage tests are made that involve all the chromosomes. It would be practically futile to undertake such work on a character of this kind that is subject to great variation in its phenotypic expression due to external conditions that are too complex to be fully controlled. Moreover the possibility of mutant modifying genes affecting so sensitive an organ appearing in the course of such an experiment would further increase the difficulties of the problem.

The second problem is much more feasible, and at several stages in the course of the work flies have been selected from the cultures that have both eyes or one eye or with no eyes to observe whether their offspring differ on the whole from those of the main line. Here again a peculiarity of the stock needs to be taken into consideration. In the earlier hatches there are only a few flies with both eyes present and these are usually small, while in the late hatched flies all may have two eyes and these may be quite large. Now if the former were more sensitive to the external conditions, they might or might not be the ones best suited for a test (depending on what was to be tested) or they might be those whose genetic make-up (if the stock were not entirely homozygous) favored the development of two eyes. In this respect, if the presence of genetic differences were being sought for, these might be better than the late-hatching flies. For, the late-hatching flies will obviously include flies with two eyes which involve all the genetic types present, etc. It is obvious from these and other considerations that the problem here is, as in all such cases, very complex. As a matter of fact, most of the selections in the positive direction were made from the earlier or middle hatchings. A few experiments were deliberately planned in which late-hatching flies were used. It may be stated that no measurable differences in the two cases was found.

Several selections towards full eye have been made. The earlier ones in the 9th, 16th, 17th, 18th, and 20th generations of select-down are given in the following records (table 21). There is no evidence in any of these cases that the first selection of the phenotype with one or two eyes present gives any difference in the offspring from the "no eyes" flies of the same stocks.

Later, a much more complete series of tests were made through three to

Table 21

Select back, one eye (out of down[9])	Both	One	None
	2	14	31
	4	19	46
Select back, both eyes (out of down [16])	**Both**	**One**	**None**
	0	0	3
	0	2	20
	0	4	10
	2	6	40
	7	17	11
	16	1	2
	0	10	18
	14	6	2
	9	14	35
	0	0	3
Select back, (out of down[16])	**Both**	**One**	**None**
	1	5	56
	4	21	30
	14	8	18
	40	27	9
Select back, 1 ♀ one eye, 2 ♂ both eyes (out of down [16])	**Both**	**One**	**None**
	0	10	13
	1	7	5
	2	2	0
Select back, both eyes (out of down[16])	**Both**	**One**	**None**
	6	28	143
	8	26	104
Select back, both eyes (out of down[16] and[17])	**Both ♀**	**One**	**None**
	11	19	77
	2	11	12
	13	9	2
	9	10	0
	6	5	3

TABLE 21—*Continued*

Select back, one eye (out of down^{17})

	Both	One	None
	0	1	31

Select back, (out of down ^{17})

	Both	One	None
B^a both eyes	12	24	77
	0	0	31
B^b one eye	0	1	40
	0	3	31
	5	9	9

Select back, both eyes (out of down^{18})

	Both	One	None
	2	3	15
	6	11	34
	11	29	119

Select back, B^c, no eye (out of down^{18})

	Both	One	None
	6	8	77

Select back, one eye (out of down^{20})

	Both	One	None
	4	12	81

Select back, both eyes (out of down^{20})

	Both	One	None
	2	6	121

Select back, (out of down^{20})

	Both	One	None
A^a no eyes	5	2	59
A^b one eye	0	14	97
A^c both eyes	1	11	108

TABLE 22

Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Both	One	None
47 no eyes	48 no eyes	....	....	....	....	....	49	4	31
43 one eye	44 one eye	45 one eye	....	....	....	....	19	53	65
43 one eye	44 one eye	45 one eye	46 one eye	....	....	....	3	32	132
43 one eye	44 one eye	45 one eye	46 one eye	....	....	....	0	20	65
			46 one eye	....	....	....	1	44	147
			46 one eye	....	....	....	12	62	158
38 one eye	39 one eye	40 one eye	41 one eye	....	....	....	32	20	30
38 one eye	39 one eye	40 one eye	41 one eye	42 one eye	....	....	8	21	16
45 one eye	46 one eye	47 one eye	48 one eye	49 one eye	....	....	8	13	29
45 one eye	46 one eye	47 one eye	48 one eye	....	....	....	39	75	87
			48 one eye	....	....	....	0	15	22
43 one eye	44 one eye	45 one eye	46 one eye	47 one eye	48 one eye	....	1	3	1
43 one eye	44 one eye	45 one eye	46 one eye	47 one eye	48 one eye	....	6	17	31
43 one eye	44 one eye	45 one eye	46 one eye	47 one eye	48 one eye	....	10	13	26

Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Both	One	None
43 one	45 one	46 one	47 one	48 no	49 no	50 no	......	....	25	34	45
43 one	44 one	45 one	46 one	47 one	48 no	49 no	......	....	0	1	13
43 one	44 one	45 one	46 one	47 one	48 no	49 no	50 no	....	4	18	63
43 one	44 one	45 one	46 one	47 one	48 no	49 no	50 no	....	15	42	96
43 one	44 one	45 one	46 one	47 one	48 no	49 no	50 no	51 no	15	49	115
43 one	44 one	45 one	46 one	47 no	48 no	49 no	50 no	....	16	33	89

Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Both	One	None
42 both	43 both	44 both	45 both	....	....	22	39	26
41 one	42 both	43 both	44 both	45 both	....	24	38	23
40 one	41 both	42 both	43 both	44 one	....	6	27	12
42 one	43 both	44 both	45 both	....	....	58	71	51
41 one	42 both	43 both	44 both	45 both	....	10	36	30
41 one	42 both	43 both	44 one	....	....	30	25	18
41 one	42 both	43 both	44 both	....	....	10	12	18

Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Gen.	Both	One	None
43 one	44 one	45 one	46 one	47 one	48 no eye	49 no eye	4	21	46
							8	13	29
							5	22	100
							12	30	66
							8	20	65
							3	14	25
							4	9	7
							4	31	44
42 one	43 one	45 one	46 one	47 none	48 none	....	39	75	87
42 one	43 one	45 one	46 one	47 none	48 none	....	0	15	22
42 one	43 one	45 one	46 one	47 one	48 both	....	6	14	29
42 one	43 one	45 one	46 one	47 one	48 both	....	4	11	5
42 one	43 one	45 one	46 one	47 one	48 none	49 both	1	7	15
42 one	43 one	45 one	46 one	47 one	48 both	....	16	10	13
42 one	43 one	45 one	46 one	47 none	48 none	49 none	12	43	36
42 one	43 one	45 one	46 one	47 one	48 both	49 both	12	0	15
42 one	43 one	45 one	46 one	47 none	48 none	49 none	0	19	55
			46 none	47 none	48 none	49 both	2	12	37
			46 one	47 none	48 none	49 none	2	10	10
			46 one	48 none	49 none	50 none	3	3	8
			46 one	47 none	48 none	49 none 50 none	7	37	59

six consecutive selections (table 22). It is evident that no change was brought about by selection of the stock at this time. In other words, it had become practically homozygous for the modifiers of eyeless.

These data from flies in the 38th to the 51st generation are typical. In some cases the flies continued to be selected for one eye. The record in each case gives the last generation. In other cases, after one or another kind of selection (indicated in the table) had been obtained, reversed selection took place one or more times. Despite the rather wide variability there is no evidence that reversed selection produced any effect.

In addition to these selections, the records (table 23) carried several generations further than the last, may be added.

TABLE 23

	Gen.	Both	One	None
No eyes for 6 generations, then one eye for 4 generations......	54th	7	50	213
No eyes for 7 generations, then one eye for 4 generations......	54th	1	11	65
No eyes for 8 generations, then one eye for 4 generations......	54th	1	16	183
No eyes for 8 generations, then one eye for 4 generations......	54th	9	45	276
No eyes for 8 generations, then one eye for 4 generations......	54th	6	19	83
No eyes for 8 generations, then one eye for 4 generations......	55th	0	18	134
No eyes for 8 generations, then one eye for 4 generations......	55th	13	55	105
No eyes for 8 generations, then one eye for 4 generations......	53rd	6	9	5
No eyes for 8 generations, then one eye for 4 generations......	55th	7	30	62
No eyes for 8 generations, then one eye for 4 generations......	55th	22	67	128
No eyes for 9 generations, then one eye for 4 generations......	56th	0	16	182
No eyes for 9 generations, then one eye for 4 generations......	55th	1	34	93
No eyes for 9 generations, then one eye for 4 generations......	56th	10	28	99
No eyes for 9 generations, then one eye for 4 generations......	56th	0	9	10
No eyes for 9 generations, then one eye for 4 generations......	55th	0	27	266
No eyes for 9 generations, then one eye for 4 generations......	55th	4	16	121
No eyes for 9 generations, then one eye for 4 generations......	55th	0	5	18
No eyes for 9 generations, then one eye for 4 generations......	56th	0	5	127
No eyes for 9 generations, then one eye for 4 generations......	56th	0	8	34
No eyes for 9 generations, then one eye for 4 generations......	56th	8	31	139
No eyes for 10 generations, then one eye for 4 generations.....	57th	0	0	9
No eyes for 10 generations, then one eye for 4 generations.....	57th	5	27	155
No eyes for 10 generations, then one eye for 4 generations.....	56th	3	25	141
No eyes for 10 generations, then one eye for 4 generations.....	57th	5	26	215
No eyes for 10 generations, then one eye for 4 generations.....	55th	0	13	157
No eyes for 10 generations, then one eye for 4 generations.....	56th	21	41	159
Both eyes for 1 gen., no eyes for 9 gen., one eye for 4 gen......	55th	0	0	12
Both eyes for 1 gen., no eyes for 9 gen., one eye for 4 gen......	55th	3	12	101
Both eyes for 1 gen., no eyes for 8 gen., one eye for 4 gen......	56th	7	20	21

In one experiment flies out of the same culture from the select-down stock in the 35th generation, of three kinds (three with two eyes; others with one eye, others with no eyes) were bred with the following results:

Parents both eyes 35th gen.			Parents one eye 35th gen.			Parents no eyes 35th gen.		
Both	One	None	Both	One	None	Both	One	None
11	53	93	1	19	59	20	44	120

There is no significant difference here in the kinds of offspring produced by flies of the different phenotypes.

In the preceding data the end results of certain selections are given. As a control, the kinds of flies in the parental cultures were recorded, in some cases for one generation only (1), in other cases for several generations (2) (3) (4). A few samples of these are given to illustrate the fluctuations shown by successive generations. These records come from the end of the experiments, usually within the 50th to 60th generation (table 24).

Table 24—*Three or more generations*

No eyes				One eye				Both eyes			
	Both	One	None		Both	One	None		Both	One	None
(1)	8	10	28	(1)	37	17	4	(1)	15	17	6
	14	21	63		11	35	42		5	10	28
(1)	37	17	4 (3rd count)	(1)	6	17	4	(1)	25	2	2
	26	15	2		18	77	60		3	40	53
(1)	6	14	4 (3rd count)	(1)	10	8	11	(1)	14	28	17
	4	4	4		2	5	14		6	5	23
(1)	13	4	4 (3rd count)	(1)	5	27	22	(1)	2	24	14
	3	22	63		4	21	65		10	52	107
(1)	18	4	2 (3rd count)	(1)	15	17	6	(1)	11	16	3
	22	23	28		1	1	18		1	2	25
(1)	2	10	15 (4th count)	(1)	8	13	28	(1)	18	16	3
	6	13	48		8	37	59		0	4	14
(1)	5	27	22 (4th count)	(1)	2	24	14	(1)	14	28	17
	22	8	0		10	52	107		6	5	23
(1)	2	10	15 (4th count)	(1)	10	8	11	(1)	6	17	15
	1	24	99		2	5	14		65	105	88
(1)	1	17	8	(1)	5	27	22	(1)	67	67	62
	5	25	74		4	21	65		25	15	2
(1)	14	18	17	(1)	6	17	15				
	13	28	104		74	105	50				
(1)	2	24	14								
	8	20	69								
(1)	5	27	22								
	26	36	64								
(1)	2	24	14 (2nd count)								
	8	20	69								
(1)	6	17	15								
	24	61	61								

TABLE 24—*Three or more generations*—Continued

Both eyes				Both eyes			
	Both	One	None		Both	One	None
(1)	5	27	22	(1)	25	18	2
(2)	*32*	8	0	(2)	37	17	4
	21	16	4	(3)	*32*	7	6
					20	33	78
(1)	*24*	10	3				
(2)	*69*	64	45	(1)	*65*	105	88
	7	14	8	(2)	6	17	13
				(3)	*146*	16	78
(1)	55	17	0		67	65	32
(2)	*16*	7	28				
	24	10	3	(1)	55	17	0
				(2)	16	7	28
(1)	*65*	112	88	(3)	24	10	3
(2)	*6*	17	17	(4)	*69*	54	45
	6	39	106		15	14	8
(1)	55	17	0	(1)	65	105	88
(2)	16	7	28	(2)	6	17	13
(3)	*24*	10	3	(3)	146	16	38
	132	91	55	(4)	*67*	65	32
					1	27	60
(1)	6	17	55				
(2)	65	105	88	(1)	55	17	0
(3)	*13*	3	0	(2)	16	7	28
	3	5	26	(3)	*24*	10	3
				(4)	*19*	22	16
(1)	6	17	55		33	57	73
(2)	95	108	58				
(3)	*65*	9	0	(1)	65	65	52
	17	36	151	(2)	0	15	50
				(3)	25	15	2
(1)	65	105	85	(4)	*38*	25	1
(2)	10	13	2		4	11	11
(3)	*55*	17	0				
	23	55	92	(1)	55	17	0
				(2)	16	7	28
(1)	6	17	15	(3)	24	10	3
(2)	65	105	88	(4)	*47*	13	3
(3)	*13*	7	0		28	38	50
	16	24	40				
				(1)	55	17	0
(1)	65	105	88	(2)	16	7	28
(2)	6	17	13	(3)	24	10	3
(3)	*146*	76	38	(4)	69	54	45
	21	13	4	(5)	*15*	14	8
					19	31	35
(1)	65	105	88				
(2)	6	17	13				
(3)	*43*	17	4				
	71	64	76				

THE NON-INHERITANCE OF AN ACQUIRED CHARACTER AS ILLUSTRATED BY EYELESS

In order that a character be "acquired" in the sense here employed it must be directly susceptible of being changed by some environmental agent, or indirectly affected by its use or disuse. It has been claimed that characters acquired in either of these ways may be or are transmitted through the germ-cells to the next generation. It might appear on first thought that those special characters that are most easily affected, or affected to a great extent would be the ones best suited to test the question of their inheritance. In fact, in most "experiments" in recent years it is this sort of material that has most often been used (salamanders and pupæ). This is, however, a questionable assumption, and there is nothing known that supports such a view, but since this sort of character is the one most likely to attract attention, it has been often chosen to carry out such tests.

Modern genetic work has shown that the problem of the inheritance or non-inheritance of acquired characters is intimately bound up with the causes of variability in any given stock. It has been amply demonstrated that internal genetic factors may sometimes produce results that are superficially indistinguishable from those produced by external agents. The present case is an excellent illustration of this relation, for, both genetic factors and environmental factors change the eyeless flies either back to full eye or to entire absence of eyes. These possibilities must be watched, and, wherever they introduce disturbing factors, the experiment must be carried out so as to detect them. The most general rule is to make the material homozygous as far as possible, and in the second place to use pedigreed material whose genetic possibilities have been tested by inbreeding over several or many generations. An effort to do this has been made in the experiment with eyeless where the material was inbred for about 60 generations. The parents in almost every generation were without eyes, yet after about 60 generation of ancestors without eyes, the same kinds of flies were produced as at the beginning, *viz.,* flies without eyes, with small eyes, and flies with larger eyes—as did the flies after the first three or four generations. The absence of eyes in the ancestors produced no change in the character of the offspring after the modifying genetic factors had once been made constant.

In the present case the flies bred from were kept without eyes or rudiments of eyes by the presence of genetic factors which were assisted by environmental agencies. This somatic absence produced no effect on the character; for, if it had done so, the eyes would either have disappeared, or have been further reduced as the selection continued. The data showed that this did not take place.

The evidence also shows that the principal gene was not changed, for whenever these flies were outbred and new eyeless stock subsequently recovered, it gave exactly the same results as did the original stock. The gene for eyeless was neither "contaminated" by outbreeding, nor was it changed by selection.

	Both	One	None
d⁷.....	16	37	70
	1	6	33
	18	22	18
	44	35	25
	53	23	6
	34	32	33
	14	55	90
d⁸.....	12	15	8
	10	33	42
	49	34	59
	38	45	127
	12	32	100
	14	37	29
	0	10	44
d⁹.....	25	27	64
	3	31	35
	21	46	112
	36	52	153
	4	19	32
	2	10	92
d¹⁰....	1	13	37
	26	20	16
	27	50	87
	34	22	31
	3	32	138
	29	30	40
d¹¹.....	6	13	12
	43	101	97
	3	13	115
	52	27	9
	5	24	121
d¹².....	6	18	32
	2	15	74
	17	49	148
	14	30	127
d¹³.....	7	24	264
	3	11	58
	0	4	30
	14	44	219
	4	27	81
	4	7	26
	37	64	140
d¹³.....	7	47	240
	4	19	46
	23	29	149
d¹⁴.....	12	10	38
	28	51	73
	28	39	110
	12	20	58
	48	34	88
	0	8	58
	7	23	16

	Both	One	None
	6	20	121
	10	32	135
	15	21	133
	5	28	135
	4	22	67
	12	37	143
	13	23	58
	11	42	148
	16	27	94
	25	31	82
	9	27	137
d¹⁵.....	20	34	161
	31	59	185
	6	11	19
	0	4	11
	0	4	32
	3	23	106
	0	17	159
	0	6	49
	0	7	27
	3	6	42
	5	15	80
	1	6	56
	16	27	42
	10	24	131
	7	28	91
	15	16	78
	9	11	28
	21	42	71
	2	16	86
	15	42	94
	11	41	191
	30	55	149
	9	24	63
	7	34	179
d¹⁵.....	7	24	127
	37	62	172
d¹⁶.....	3	29	179
	50	64	121
	8	28	92
	14	44	116
	3	3	23
	2	8	67
d¹⁶.....	39	47	110
	3	29	131
	8	30	105
	14	22	73
	8	15	75
	21	24	46
	11	68	220
	22	44	131
	2	6	19
	11	39	181

	Both	One	None
	17	54	110
	1	8	57
	6	14	21
d¹⁶.....	12	51	174
	11	25	136
	0	16	53
	0	24	129
d¹⁶.....	1	17	16
	1	23	148
d¹⁷.....	1	6	14
	0	10	76
	0	6	86
	8	9	56
	3	17	158
	4	20	68
	9	32	170
	5	30	129
	3	17	50
	19	64	188
d¹⁷.....	1	10	23
	49	85	84
	3	21	147
	0	10	69
	7	15	39
	4	18	120
	15	44	159
	0	15	211
d¹⁷.....	17	33	142
	0	15	133
	8	31	168
	10	14	138
	6	15	47
	4	17	83
	2	13	67
	6	5	57
	4	8	79
	0	9	84
	17	41	129
	13	41	197
	21	65	257
	4	11	133
	3	5	125
	6	28	85
	5	33	158
d¹⁷.....	0	0	14
	2	3	108
	4	9	17
	20	9	2
	5	1	0
	5	7	0
	2	4	5
	9	11	2
	0	1	1

	Both	One	None
d^{18}.....	2	16	82
	3	18	128
	1	19	71
	10	28	70
	2	20	158
	7	11	6
	1	14	54
	21	55	119
d^{18}.....	0	6	60
	0	3	10
	0	4	4
	1	6	12
	6	7	7
	24	18	8
	10	3	3
	5	9	9
d^{18}.....	40	110	337
	46	56	113
	16	66	195
	4	16	62
	10	63	172
	4	20	186
	3	6	110
	7	25	155
	9	33	79
	14	16	14
	5	11	60
	0	11	77
	3	13	69
	8	22	120
	5	15	47
	4	37	233
	9	23	116
	2	8	100
	18	38	230
	26	40	129
	18	48	200
	5	14	87
	6	39	148
	1	14	125
	3	17	59
	1	10	34
d^{18}.....	1	4	54
	7	18	59
	17	55	38
	14	27	9
	11	11	5
	8	8	6
	3	7	0
	16	9	4
	6	3	0
d^{18}.....	2	5	52
	4	28	151
	11	22	26
	29	33	22
	10	16	5
	0	13	5
	0	5	2
	3	6	3
	2	2	2

	Both	One	None
d^{19}....	1	5	49
	0	3	25
	4	17	46
	8	8	9
	3	3	1
	7	5	3
	0	4	0
d^{19}.....	0	7	51
	0	7	22
	0	0	4
	9	16	11
	7	21	9
	24	23	3
d^{19}.....	0	9	68
	1	3	17
	29	25	7
d^{19}.....	24	46	173
d^{19}.....	5	10	72
	2	19	85
	1	18	78
	1	9	80
	8	30	50
	0	11	150
	30	49	141
	2	12	118
	2	11	87
	15	25	74
	3	13	78
	0	6	26
	10	32	113
	4	24	181
	1	18	204
d^{20}.....	1	6	54
	3	8	21
	6	6	7
	12	8	1
	2	12	6
	22	18	10
	0	2	1
	2	1	2
	0	2	3
d^{20}.....	17	31	180
	1	11	89
	0	5	56
	4	13	107
	26	66	220
	3	63	178
	16	36	60
	5	25	93
d^{20}.....	0	12	71
	1	13	21
	0	2	13
	2	9	36
	5	7	10
	18	8	6
	1	12	25

	Both	One	None
d^{20}.....	1	8	60
d^{20}.....	0	17	91
	7	23	46
	17	31	75
	14	26	111
	13	17	71
d^{21}.....	0	1	20
	0	2	25
	0	0	29
	2	1	42
	5	3	33
d^{21}.....	0	5	6
	1	12	115
	3	17	35
d^{21}.....	0	15	119
	37	60	131
	24	40	192
	2	5	39
	0	5	150
	12	60	106
	6	42	172
	0	4	34
	1	8	153
	5	29	136
d^{21}.....	0	2	11
	3	5	40
	1	3	18
	12	34	25
	3	1	2
d^{21}.....	0	6	61
	2	12	61
	6	13	5
	6	7	1
	8	0	0
d^{21}.....	9	58	223
d^{22}.....	5	34	146
	5	29	74
	4	20	56
	0	4	36
	5	8	87
	4	13	90
	2	7	56
d^{22}.....	24	68	168
	4	30	187
	20	55	102
	2	15	63
	0	7	69
	25	84	189
	13	41	168
	35	121	324
	16	20	83
d^{23}.....	13	20	87
	54	114	298

	Both	One	None
d^{23}.....	18	42	112
	13	70	245
	5	41	133
	4	8	52
	0	3	118
	0	17	114
	7	15	16
d^{23}.....	5	20	45
	0	9	65
	2	38	100
	20	48	141
d^{24}.....	5	34	150
	10	21	107
	1	18	167
	1	12	80
	0	7	105
	5	26	127
d^{25}.....	6	36	119
	15	12	145
d^{26}.....			
d^{27}.....			
d^{28}.....	6	22	120
d^{29}.....	56	98	230
	28	30	69
	6	9	8
d^{30}.....	10	44	103
	11	30	92
	6	9	8
	24	31	67
d^{31}.....	0	10	83
	1	2	11
	0	14	27
	3	5	21
	0	11	15
	1	9	18
	0	1	2
d^{31}.....	21	68	186
	25	48	98
	6	14	8
	17	43	54
d^{32}.....	10	40	79
	5	16	95
	7	29	79
	13	37	72
	2	12	40
	0	3	33
	6	27	96
	5	15	71
d^{33}.....	22	33	44
	0	19	87
	3	13	44

	Both	One	None
d^{33}.....	7	30	85
	1	13	62
	48	90	196
	12	34	143
	4	22	67
	28	75	165
d^{34}.....	8	19	25
	16	54	110
	0	11	89
	15	17	26
	46	39	50
	1	8	94
	8	52	158
	0	12	48
	8	27	139
	40	74	131
	7	18	59
	6	24	36
	9	31	140
d^{35}.....	44	35	30
	0	9	85
	1	9	49
	1	19	97
	1	4	53
	33	46	121
	0	8	97
	31	38	125
	2	10	87
d^{36}.....	0	6	26
	6	29	91
	1	24	58
	0	9	37
	2	8	40
d^{37}.....	50	81	191

	Both	One	None
d^{38}			
(C) both eyes	8	26	143
(A) no eyes	36	61	236
(B) one eye	14	45	215
both eyes	2	10	55
one eye	5	36	127
no eye	11	53	110
	17	64	120

	Both	One	None
d^{39}.....	5	10	81
	9	35	102
	3	23	97
	2	17	63
	9	74	196
	23	46	73
	19	52	227
	15	55	219
d^{40}.....	2	16	100
	1	17	102
	3	22	155

	Both	One	None
d^{40}.....	1	15	226
	3	23	155
	9	24	147
	11	26	70
d^{41}.....	4	15	31
	6	19	70
	25	40	57
	19	50	165
	7	38	111
	21	48	202
	7	41	148
d^{42}.....	30	52	39
	22	74	231
	22	33	136
	36	70	164
	8	38	125
d^{43}.....	4	18	42
	1	15	35
	17	47	61
	44	49	26
	18	45	61
	75	83	45
	54	78	37
	23	23	1
	6	30	45
	18	40	27
	44	60	42
d^{44}.....	1	11	34
	18	16	7
	28	25	24
	34	73	73
	42	53	13
	56	93	81
	23	21	41
	51	44	28
	10	12	18
	30	25	18
	6	27	12
d^{45}.....	28	20	4
	13	33	47
	20	34	22
	34	48	25
	37	38	19
	35	26	18
	22	22	2
d^{45}.....	6	10	13
	24	38	23
	58	71	51
	19	53	65
	22	39	26
	10	36	30
d^{46}.....	28	50	30
	19	19	7
	3	6	0
	73	65	22
	25	27	17
	12	62	158

	Both	One	None
d^{46}.....	1	44	147
	3	32	132
	0	20	65
	10	14	8
d^{47}.....	6	6	8
	5	17	4
	6	14	5
	8	62	62
d^{48}.....	3	6	7
	4	9	6
	2	1	2
	39	75	87
	0	15	22
d^{48}.....	1	3	1
	6	17	31
	6	14	29
	4	11	5
	16	10	13
	10	13	26
d^{49}.....	3	1	1
	2	10	10
	6	21	30
d^{49}.....	0	2	9
	0	19	55
	2	12	37
	1	6	15
	4	21	46
	8	13	29
	5	22	100
	12	30	66
	8	20	65
	3	14	25
d^{49}.....	4	9	7
	4	31	44
	1	7	15
	0	1	13
	12	0	3
	12	43	36
d^{50}.....	3	3	8
	7	37	59
	4	18	63
	15	42	96
d^{50}.....	16	33	89
	25	34	45
d^{51}.....	19	52	61
	15	49	115
	2	33	107
d^{52}.....	1	3	11
	18	57	122
	0	2	58
	0	16	136
	3	9	47
	1	14	111
	17	71	192
d^{53}.....	8	29	173
	3	26	140
	38	42	77
	0	4	34
	4	11	64
	3	10	78
	3	25	119
	5	37	269
	6	9	5
d^{54}.....	1	19	168
	9	54	209
	5	16	100
d^{54}.....	0	2	9
	2	15	44
	1	16	183
	9	45	276
d^{54}.....	6	19	83
	1	11	65
d^{54}.....	7	50	213
	2	8	82
d^{55}.....	0	18	134
	13	55	105
	7	30	62
	1	34	93
	0	13	157
	0	27	266
d^{55}.....	4	16	121
	0	5	18
d^{55}.....	22	67	128
	3	12	101
d^{56}.....	0	16	182
	3	25	141
	21	41	159
d^{56}.....	1	2	4
	2	9	32
	0	3	53
	10	28	99
	0	9	10
	0	5	127
d^{56}.....	0	8	34
	8	31	139
d^{56}.....	7	20	21
	0	4	29
	15	47	115
	26	30	32
d^{56}.....	9	13	16
	3	29	256
d^{57}.....	5	27	155
	3	18	60
	5	32	159
	2	1	32
	10	70	506
	5	29	151
	9	26	57
d^{58}.....	9	38	207
	7	13	125
	12	52	172
	0	7	119
	3	33	172
d^{59}.....	1	30	178
	1	27	421
	5	20	121
d^{60}.....	3	27	165
	10	64	281
	0	27	216
	2	15	47
	4	33	258

VI

DATA RELATING TO SIX MUTANTS OF DROSOPHILA

By T. H. Morgan

With six text-figures

DATA RELATING TO SIX MUTANTS OF DROSOPHILA

By T. H. Morgan

INTRODUCTORY

The six mutants and mutational changes, described in the following pages, appeared several years ago. After locating approximately their genes in the chromosomes, three of them have been discarded owing to their low viability. The modifiers for forked and for scute were also allowed to go, but have reappeared from time to time in other stocks. Their chief interest lies in the rather unusual character of genes that transform the recessive mutants forked and scute back to normal phenotype. Should such a gene appear in an experiment in which scute was present it would play havoc with the ratios unless detected. There is also a wider evolutionary interest in modifications of this kind as has been pointed out in the recent paper of R. A. Fisher (*American Naturalist* 1928).

CHANGE OF A RECESSIVE MUTANT CHARACTER FORKED INTO THE WILD PHENOTYPE

In certain crosses when forked flies only were expected in certain classes, a few of the expected forked offspring had normal bristles. There were three explanations of such an occurrence that seemed possible. The mutant gene may have reverted to normal, or some other gene had mutated whose effect was to change forked to normal bristles, or else the character forked fluctuates so widely in this stock that at one extreme it is indistinguishable from the normal. The last possibility, in so far as internal factors are concerned, is closely related to the second possibility, since certain genetic complexes might cause wider variability than other genetic complexes, etc.

The following evidence shows that the first suggestion is not correct because the changed-over individuals, when tested, produce some or even all forked offspring. The last suggestion is not true for the forked stock itself, because all the flies in such stock are distinctly forked. The second suggestion by exclusion may seem then to furnish an explanation, and the evidence, to be adduced, supports this view. The change was first observed in crosses of attached-X (double yellow) females to forked Bar males. The sons in such a cross all arise from a Y-chromosome egg fertilized by the X-sperm of the father. The sons, therefore, receiving their single X from the father are expected to show the sex-linked character of the father (X-patroclinous). In cultures in which the father was forked Bar, a few Bar males appeared that were not forked as the data recorded in table 1 show.

Some of the forked males in this stock were sibs of F_1 flies that had given not-forked in the preceding generation. There were also several cultures in which all the males were forked that are not here recorded.

In other crosses the males were forked Bar fused. These gave the results shown in table 2.

Here also certain cultures gave only forked fused Bar males and are not reported. There are three flies that are recorded as Bar. They can not be crossovers between Bar and fused since this would have to occur in the male.

TABLE 1

Attached-X yy ♀ by forked Bar ♂		
yy ♀	f B ♂	B ♂
246	221	10
136	210	2
136	190	2
186	226	6
100	91	2
11	15	3
41	43	9
97	89	1
30	30	1
82	40	1
237	303	5
273	151	4 (near forked)
76	137	1
283	326	9
183	239	6
166	179	5
82	84	2
43	67	1
130	159	6
146	137	11
145	141	y ♂ het. B ♀ N ♂ XXX ♀ f B ♀ 1 3 2 5 2
273	151	4
184	174	19 (+ 4 near forked) XX 2

At different times the not-forked males were tested by breeding to attached-X yy-females of the same stock (as a rule to their sisters). If the forked gene had reverted to wild type, the sons would be not-forked.

TABLE 2

Attached-X yy ♀ by forked Bar fused ♂				
yy ♀	f B fu ♂	fu B ♂	B ♂	XXX ♀
183	126	1	. .	6
268	224	2	. .	. .
92	93	0	2	. .
161	101	0	1	. .
149	92	1	. .	. .
169	158	2	. .	. .
76	100	2	. .	. .
193	93	4	0	1
96	72	4	. .	. .
132	124	31	. .	10

If the forked gene had not reverted, but modifying factors were present, the result would depend on the number and distribution of these factors in the father and mother. The results obtained are shown in table 3.

TABLE 3

Attached-X yy ♀ by not-forked ♂, sibs of forked males.

yy ♀	f B ♂	B ♂	XXX ♀	y ♂	yy ♀	f B ♂	B ♂	XXX ♀	y ♂
81	100	13	..	..	121	95	..	..	..
82	48	35	..	..	11	13	..	..	..
77	69	0	..	..	14	2	6	..	..
31	38	0	..	..	45	26	..	..	..
41	56	..	..	..	105	123	12	..	..
81	60	13	..	..	33	13	..	..	..
85	80	..	..	..	65	69	5	..	..
85	67	..	..	..	24	29	..	..	..
36	46	..	..	..	80	54	24	..	..
69	72	..	..	..	20	14	6	..	..
49	23	..	..	..	108	49	45	..	..
18	12	12	..	..	157	161	..	8	..
86	68	34	..	..	49	26	29	..	..
41	25	7	..	..	20	22	2	..	..
80	95	17	..	..	180	165	..	..	1
40	39	26	..	..	35	31	13	..	..
76	23	6	..	..	60	32	..	..	..
221	140	5	..	..	44	44	..	..	..
75	22	0	..	..	..	..	..	..	..

In 17 cultures there were no males that were not-forked which means that the not-forked fathers were genetic forked males. In 19 cultures there were some not-forked males, which means that the factors for changed forked were present, and, if recessive, were present in homozygous condition. That the latter is the correct interpretation was shown by continued inbreeding of not-forked brothers of males that were forked (from the same culture). The results are given in table 4. The material of table 4 is from later records kept by Miss E. M. Wallace to whom the stocks were turned over for inbreeding. In each generation not-forked Bar males were bred to attached-X yy sisters. After the fifth generation one line only was followed out. In the 17th generation the stock nearly ran out, and the single not-forked male was bred to other stock (attached-X yy). Among the offspring of the 18th and 19th generations a few males only were not-forked and these doubtfully so. In later generations the number of not-forked males was not appreciably different from before.

In practically all these cultures, with two exceptions, not-forked males were present. The total of not-forked males was 2,140 and that of the forked males 6,243. There were 8,663 females, which is practically the same number as that of the males. This means that the not-forked factor or factors has not decreased the number of males. The result appears to mean that the not-forked character overlaps forked so that many or all (?) forked males belong to one class. After this number of generations the stock would be expected to be homozygous for the factors producing not-forked. The fact, that all the males are not like this, seems to mean that on the average not more than a third of the males of this kind are phenotype not-forked.

There are a few records that give an unusual result. One, not-forked Bar fused male (with yy ♀) gave 16 yy ♀, 17 not-forked B ♂. Another, not-forked Bar male (by yy ♀), gave 44 yy ♀ and 53 not-forked males.

TABLE 4

Attached-X yellow ♀ by not-forked B ♂ (carrying the modifying genes)

Generation	yy ♀	f B ♂	not-f B ♂	het. B ♀	N ♀	XXX ♀	y ♂	N ♂	other mutants
1	514	408	54	1	1	0	1		
2	1016	809	312	..	..	3	..	..	"curly" y ♀
3	695	517	259	..	..	3	..	..	1
4	390	315	168	..	..	..	1	..	
5	96	58	59	..	..	1	..	..	
6	92	140	0	..	..	..	..	..	
7	47	42	8	..	..	..	..	..	♂ + ♀ "tumor" queer wings
8	136	214	52	..	..	..	..	..	20
9	151	217	0	2	0	0	0	0	38
10	544	546	13	..	..	..	..	..	
11	120	103	14	..	..	..	..	..	
12	301	200	12	1	..	..	..	..	
13	96	79	18	..	..	..	..	..	
14	48	27	3	0	0	1	..	..	
15	114	141	14	..	..	..	..	..	
16	32	17	4	..	..	..	..	..	
17	4	1	1	(yy ♀ from stock by not-f B ♂)					broad B ♂
18	103	154	4?	..	..	13	..	..	1
19	189	227	13?	..	..	4	..	..	
20	118	110	15	..	..	0	..	..	
21	345	253	80	..	..	6	..	..	
22	103	84	35	1	..	2	..	..	
23	492	397	129	4	..	4	2	..	
24	483	359	194	6	..	1	1	..	
25	315	208	83	0	0	10	1	..	"ultra" B ♂
26	213	188	78	0	0	3	0	..	2
27	400	310	100	4	0	0	4	..	
28	101	72	33	..	..	..	..	..	
29	152	120	55	..	..	..	..	..	
30	157	94	51	..	..	..	..	..	
31	293	283	91	0	0	10	..	..	
32	190	148	44	0	0	0	0	..	
33	262	211	86	0	0	5	0	1	
34	149	162	54	..	..	2	..		
35	30	29	4	..	..	..	..		
Totals	8,491	7,243	2,140	19	1	68	10	1	62

Another not-forked Bar fused ♂ (by yy ♀) gave 65 yy ♀ and 44 not-forked Bar fused males. Similarly in the next generation some of the last males were bred to the *original* stock (attached-X yy ♀) and gave 58 yy ♀ and 62 not-forked Bar fused ♂ (plus 2 xxx ♀).

Another Bar fused male (by yy ♀ out of yy by f B fu) gave 37 yy ♀ and 32 Bar fused males.

In another culture not-forked Bar fused males were used that for two preceding generations had also not had any not-forked sibs. It gave 116 yy ♀ and 81 not-forked Bar fused ♂. Similarly a culture of not-forked Bar fused gave 180 yy ♀ and 165 not-forked Bar fused ♂ (plus one yellow male and 63 xxx ♀). The sons (not-forked Bar fused) bred to sisters (yy) gave 51 yy ♀ and 20 not-forked fused ♂.

There are two or three possibilities to be considered.

(1) That the forked gene has reverted to normal.
(2) That contamination by wild stock occurred.
(3) That these particular stocks had become pure for some other modifier (gene) that changed forked to normal.

These possibilities may be considered in reverse order.

(3) That this explanation is not correct was shown by breeding the not-forked male to ordinary attached-X yy-females. The sons were all not-forked. Only a new sex-linked factor suppressing forked could give such a result. That none such is present is shown by breeding to forked stock (see below).

(2) That the result was not due to contamination is practically certain because both Bar and fused were present in several cases. It is true such stock may have been in the laboratory at the time, but that just this sort of contamination occurred and no other in any of the many experiments is most improbable.

(1) That the change was due to reversion seems almost certain. Out-bred in one case to attached-X's of other stock only not-forked sons were produced. Out-bred to forked^5 stocks the females were not-forked and the sons forked at the locus for f, showing that the not-forked males had a wild-type gene. Thus forked^5 virgin ♀ bred to not-forked B fu ♂ gave normal females and forked^5 ♂. In another test there were 130 normal daughters and 115 forked^5 sons. These F_1 normal daughters were back-crossed to forked^5 males. These daughters should have one forked not-Bar X-chromosome and another X that is Bar and not-forked (to be tested). The offspring confirm this analysis thus:

Females	Males			
	f^5	B	f B	N
131	78	53	0	2
128	64	34	..	..
95	56	45	..	..
37	16	21	..	..
192	72	112	..	..
66	29	23	..	..
69	35	40	..	..

These results are consistent with the view that "reversion" had taken place in the forked gene in the original chromosome carrying Bar also.

In two other cultures, but not out of the same series as the last, a different result was obtained, but here the females were forked:

Females forked	Males		
	f^5	f B	B
61	25	31	..
74	50	35	..
133	85	78	5
24	7	..	1
164	95	42	2

In these cases the original not-forked male was only a changed-over forked that reappeared in the next generation as forked Bar except in eight cases when the forked did not appear with Bar.

The conclusion that "reversion" sometimes occurs is borne out by other cases of the same sort in forked found by L. V. Morgan who supplies the following records:

> A Star Dichæte Hairless eosin cut forked over forked Bar female bred to yellow black male gave *one not-forked son* that was also Star Dichæte. He was mated to a stock forked female and gave wild-type daughters and (as expected) forked sons. One of these daughters, that was also Dichæte, was mated to a 7-ple male (including forked). The daughters were 30 forked and 35 not-forked: the sons were 16 forked to 21 not-forked. Some of the not-forked daughters were mated to not-forked sons, and four pairs of homozygous not-forked flies were carried on for several generations. No forked flies appeared.
>
> The same original stock, six months later, gave *a not-forked son* from a forked mother, which, when tested by forked, gave not-forked daughters.
>
> A third time forked mutated to not-forked in another marked stock. This stock had a lethal, near forked. Later on, when a not-forked stock had been obtained, forked reappeared. This suggests possibly that a piece had been translocated to not-forked and later, when forked reappeared, the piece was lost.

Later work has raised some further questions concerning this partial suppressor of forked. It has been suggested that it may be the same factor as that to be described in the next section which suppresses scute. The suppressor of scute acts as an all or none condition of scute, while the present suppressor of forked acts as a partial suppressor only. If the scute suppressor is the same as one found later by Bridges, that also suppresses hairy wing and cut, still another question arises. In Bridges suppressor the homozygous females are infertile. If the same holds here also then stock pure for the suppressor for forked could not be obtained since the fertile female would always be heterozygous. Nevertheless, the evidence still indicates that the suppressor for forked does not always give a sharp separation into forked or not-forked, but some flies, homozygous for the suppressor, may show the forked condition.

CHANGE IN THE RECESSIVE CHARACTER SCUTE TO NORMAL TYPE

The same dual situation that was found in the case of forked turned up also in another experiment with the recessive mutant scute. Here again the study of a modifying factor, that changed a recessive character back to normal, led also to the detection of the presence of not-scute flies in the same stock due either to their presence from the start, or to contamination, or to the mutation of scute back to wild type.

In both forked and scute the coincidence was not, I think, in any way connected with the effect of the selection as such, but rather to the way in which the problem was studied,[1] which led quickly to the exposure of the recovered mutant almost as soon as it appeared in a culture. In both cases attached-X females were bred to males showing the variable sex linked character. If reversion should occur in a male some of the sons of such a male would be changed and arouse suspicion. This is what actually happened.

---

[1] There is no evidence as far as I know that a gene so "weak," whose effect is easily changed by modifying factors, is more likely to revert than a "stronger" gene.

The mutant scute is characterized by the absence of certain bristles, particularly the posterior scutellars, but also the ocellars and postverticals. In certain experiments, in which male flies that had apricot eyes (w^a) and scute bristles were bred to attached-X females (yy), it was observed that some of the sons, all of which should be apricot scute, were apricot not-scute, *i. e.*, they had to all appearance normal bristles. Some of the data are given below.

Scute apricot ♂ by yy ♀

yy ♀	sc w^a ♂	w^a ♂	XXX ♀
18	14	11	. .
33	21	3	. .
95	59	22	4
22	5	14	. .
92	62	6	11
42	48	4	2

From these and other similar cultures some of the not-scute males were bred to yy-females, usually sisters from the same cultures. The following results were obtained:

Apricot (not-scute ♂) brothers of scute ♂ by yy ♀

yy ♀	sc w^a♂	w^a♂	XXX ♀	y♂
46	41	22	6	6
63	47	25	. .	. .
64	43	25	(plus white eye	mutant)
89	37	26	1	1
67	96	8	14	. .
18	14	11	. .	. .
39	12	18	. .	. .
19	12	13	. .	. .
18	14	11	. .	. .
45	22	15	. .	. .
33	21	3	. .	. .
30	13	16	. .	. .
30	15	2	. .	. .
21	9	5	. .	. .
60	33	20	. .	. .
41	23	11	. .	. .
93	47	15	. .	. .
19	12	13	. .	. .
105	77	6	5	. .
40	27	18	. .	. .
37	8	18	. .	. .

In several cases the not-scute males (having scute brothers) were selected for two or more consecutive generations. The results follow:

w^a ♂ by yy ♀ = w^a (plus sc w^a) ♂ by yy ♀

	yy ♀	sc w^a ♂	w^a ♂
Twice	28	24	26
	20	13	4
Three times	87	95	6
	42	31	20
	39	11	20
	37	26	3
	157	70	33
Four times	66	32	25

No noticeable change in the ratios occurred, indicating (since the sex ratios remain constant) that the recessive modifier, changes a certain number of the scute flies over to not-scute. In addition the following mixed generations may be recorded when sometimes apricot (w^a), sometimes the other type was selected.

	yy ♀	sc w^a ♂	w^a ♂	XXX ♀
yy ♀ by w^a; by sc w^a; by w^a; by w^a; by w^a	112	60	34	..
yy ♀ by w^a; by sc w^a; by w^a; by w^a	32	11	16	..
yy ♀ by w^a; by sc w^a; by w^a; by w^a	78	71	22	..
yy ♀ by w^a; by sc w^a; by w^a	106	75	28	6
yy ♀ by w^a; by sc w^a	114	84	11	6
yy ♀ by w^a; by sc w^a; by w^a; by w^a	116	53	38	..
yy ♀ by w^a; by sc w^a; by w^a	60	29	34	..
yy ♀ by w^a; by sc w^a; by w^a; by w^a; by w^a	40	17	22	5
yy ♀ by w^a; by sc w^a; by w^a; by sc w^a	54	39	6	..

These results are the same as the last, showing that the outcome is the same whether the scute or not-scute male is used. This is interpreted to mean that these stocks were pure for the modifier that changes over only a certain number of the males, sometimes half (or even a few more than half) but usually less than half.

In one experiment five suppressed scute males were mated to yy from stock, giving 106 yy females and 97 scute apricot males.

APPARENT REVERSION OF SCUTE TO WILD TYPE

Almost from the beginning some apricot not-scute males turned up that gave with yy females only not-scute males which bred true to not-scute afterwards. Evidently from the start there were mixed in with the phenotypic not-scute males a few genetic not-scute males that were also apricot. A few records will suffice for those cultures where only not-scute apricot males were present (page 179).

That these cases were not due either to the realization of only the phenotype for not-scute in the presence of the scute gene was shown by carrying some of the stocks further when they continued to give only not-scute (normal) males, as well as by the breeding tests that failed after long inbreeding to produce any other result. Moreover these not-scute males appeared early in the experiment and continued only in certain lines selected for not-scute among brother scutes. It is therefore probable that with the modifier for scute suppression, normal bristled flies were present in the stock at the beginning and selection served to separate them. The latter type, thereafter, bred true; the former only in the sense that phenotypically the homozygous strain gives both scute and not-scute flies due to fluctuation that is not-genetic.[1] Tests made later both in pair and in mass cultures revealed the presence of the modifier still in the original stock, but not any evidence of reverted scute.

yy ♀	w^a ♂	yy ♀	w^a ♂	yy ♀	w^a ♂
28	43	46	30	60	60
37	25	70	72	118	234
76	146	65	87		
37	14	91	122		
28	17	91	139		
4	16	17	17		

LOCATION OF MODIFIER FOR SCUTE

The relation of the modifier to scute is brought out in a cross between apricot suppressed scute male and wild type female. The F_1 flies were wild type and gave when inbred:

Normal ♀	Normal ♂	scute apricot ♂	apricot ♂	scute ♂
91	52	33	3	1
176	71	37	14	..
205	76	69	6	..
173	85	64	15	1
122	53	33	11	..
767	337	236	49	2

There are about one-fifth as many not-scute apricot as scute apricot; while one-third is expected on a single factor for suppressed scute. The other experiment indicates that the lacking not-scute apricot flies are in the scute apricot class since all suppression is not accomplished by the modifier. The two scute flies are cross-overs between scute and apricot.

In order to locate the suppressor gene an experiment was devised that gave its approximate location with respect to Dichæte.

(1) Males from scute stock were bred to Curly Dichæte to get Dichæte females heterozygous for scute.

---

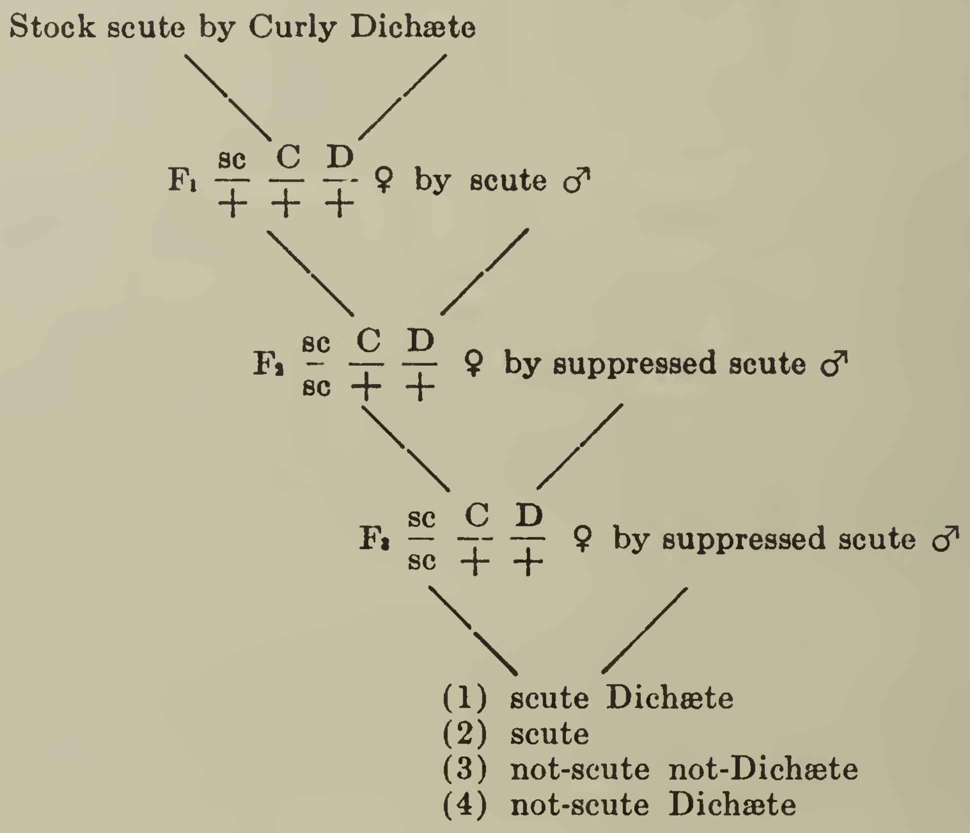
[1] It may seem probable therefore that the apricot scute stock which was not pure for scute, contained some not-scute apricot individuals rather than that such had reverted to normal.

(2) The Curly Dichæte daughters from (1) were bred to stock scute males again to get scute Curly Dichæte females.

(3) Scute Curly Dichæte females from (2) were then bred to suppressed scute males to get scute Curly Dichæte daughters heterozygous for the scute suppressor.

(4) These were then bred to suppressed scute males and the offspring classified into four classes, *viz.*, scute, Dichæte scute, not-scute and not-scute Dichæte.

The course of the matings described above is given in the following diagram.

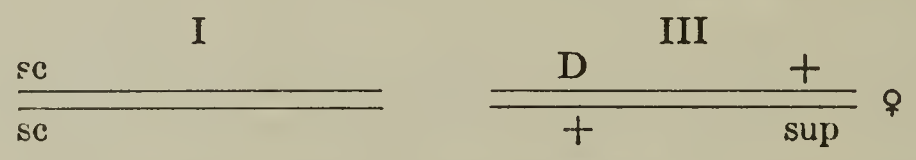

Stock scute by Curly Dichæte
$F_1\ \frac{sc}{+}\ \frac{C}{+}\ \frac{D}{+}$ ♀ by scute ♂
$F_2\ \frac{sc}{sc}\ \frac{C}{+}\ \frac{D}{+}$ ♀ by suppressed scute ♂
$F_3\ \frac{sc}{sc}\ \frac{C}{+}\ \frac{D}{+}$ ♀ by suppressed scute ♂
(1) scute Dichæte
(2) scute
(3) not-scute not-Dichæte
(4) not-scute Dichæte

The F_4 data are given in table 1.

As stated above, the Curly flies were disregarded in the final count and all the flies were separated into four classes. There were 3,321 + 422 = 3,743 flies that were scute, and 3,034 + 420 = 3,454 that were not-scute. Equal numbers are expected (if the culture is pure for scute) provided scute is *invariably* suppressed as seems to be the case.

The same data show that the location of the modifier is in the third chromosome. For example, the third chromosome pair in the mother had Dichæte in one member of the pair and the scute suppressor in the other.

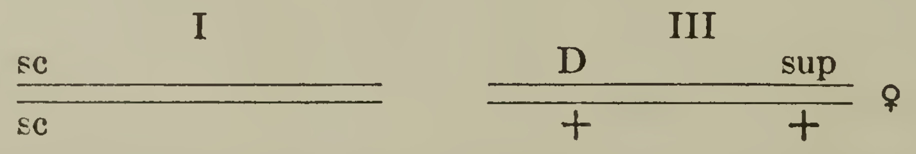
(A)



The two classes of non-crossovers are Dichæte and suppressed scute (not-scute).

When crossing-over takes place the two third chromosomes become:

(B)



The two crossover classes are, Dichæte (suppressed scute) and not-Dichæte (not-suppressed scute). Since the male that was mated to the female had the two recessive genes involved (not-Dichæte and suppressor of scute) the four classes of gametes of the female are revealed in table 1, giving:

(C)

Non-crossovers
- Dichæte scute $\dfrac{D \qquad +}{+ \qquad sup}$
- not-Dichæte sup. scute $\dfrac{+ \qquad sup}{+ \qquad sup}$

Crossovers
- Dichæte sup. scute $\dfrac{D \qquad sup}{+ \qquad sup}$
- not-Dichæte scute $\dfrac{+ \qquad +}{+ \qquad sup}$

TABLE 1—F_3 *scute Curly Dichæte by suppressed scute*

Scute		Not-scute	
Dichæte	Not-Dichæte	Not-Dichæte	Dichæte
30	4	32	2
91	3	71	5
162	21	185	17
68	7	59	5
68	2	71	5
58	13	33	8
100	12	91	7
87	11	57	10
70	13	81	3
121	15	124	12
22	2	39	4
105	9	108	8
99	15	85	15
19	7	14	2
102	18	107	17
33	5	32	2
184	11	151	18
(a) 142	35	153	66
(b) 152	25	114	62
80	15	58	12
121	10	96	6
78	5	67	8
112	10	90	10
114	10	111	16
64	11	56	5
46	2	38	0
41	3	36	4
67	4	46	1
(c) 61	19	21	2
(d) 52	5	93	27
71	11	60	3
63	12	58	4
105	10	95	8
80	4	43	4
108	23	109	18
39	7	60	2
73	11	85	11
132	10	112	4
101	12	93	7
3,321	422	3,034	420

Dividing the sum of the crossovers (422 + 420=842) by the sum of all the flies (7,197) gives 12 per cent crossing over.[1]

The further history of the scute modifier stock is given in table 2. In each generation the males that were phenotypically not scute (pure for the modifier) were bred to double yellow females. The male offspring were scute and not-scute. The females must have been heterozygous for scute suppressor.

Table 2—*Double yellow ♀ by apricot not-scute ♂ (yy ♀ × w^a not sc ♂)*

No. of gen.	yy	w^a scute ♂	w^a not-sc ♂	XXX ♀	N ♀	w^a ♀	y ♂	w^a sc ♀	gyn.	other mutants
7	521	233	268	1	1	1	..	..	..	..
8	419	249	175	5	2	0	..	..	..	..
9	195	177	146	5	0	1	..	..	..	1
10	611	303	195	3	3	1	..	..	..	..
11	288	182	118	0	0	2	1	..	..	..
12	347	217	143	1	0	1	..	..	..	..
13	261	129	91	..	..	..	..	..	..	..
14	51	27	16	..	..	..	..	..	..	..
15	110	51	44	..	..	..	..	..	..	..
16	190	119	47	0	2	..	..	..	..	..
17	327	116	133	0	0	1	1	1	..	..
18	157	138	72	..	..	..	1	..	..	..
19	248	163	96	0	1	0	1	..	..	..
20	228	117	82	..	..	..	..	..	..	..
21	92	43	37	..	..	..	..	..	..	..
22	163	87	48	..	..	..	..	..	..	..
23	47	11	9	..	..	..	..	..	..	..
24	197	105	54	0	0	0	0	1	..	..
25	68	43	26	..	..	..	..	..	..	..
26	16	4	1	..	..	..	..	..	..	..
27	...	...	...	..	..	..	..	..	..	..
28	95	49	26	0	0	0	0	1	..	..
29	231	124	58	..	..	..	..	..	..	1
30	152	89	52	..	..	..	..	..	..	..
31	216	156	57	..	..	..	2	..	..	..
32	390	220	130	..	..	..	1	..	1	..
33	349	206	92	0	1	0	1	2	..	..
34 35	531	356	192	0	2	1	..	..	..	..
36	128	86	46	..	..	..	..	..	..	..
37	12	11	4	..	..	..	..	..	..	..
38	33	35	9	..	..	..	..	..	..	..
39	81	52	33	0	1	..	..	..	..	..
..	6,754	3,898	2,500	15	13	8	8	5	1	2

In addition to the regular classes the table gives also records of exceptions, a few mutants and one gynandromorph.

Suppressed scute females were not tested since the cultures were carried on by using attached-X females. If they are sterile, the modifying factor is probably the same as that reported by Bridges (Carnegie Inst. Year

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[1] In table 1 there were four cultures (indicated by (a) (b) (c) (d)) where the ratios depart rather widely from the rest of the records. I have hesitated to omit them from the general result because there was no internal evidence of any contamination or other outside influence. Their presence affects the calculation so little as to be practically negligible.

Book No. 22, 1923), for in Bridges' line the homozygous females are sterile. His suppressor acts also as a recessive suppressor for hairy-wing and for cut. It gives all or none results, *i. e.,* there is no overlap of homozygous suppressed scute and scute. The gene lies approximately in the same locus in the third chromosome as that described above.

MUTANT NO-WINGS

A mutant that was entirely without wings appeared in a certain culture. No offspring were obtained from it but the same stock that produced it continued to produce a few no-wing flies. After several attempts a stock was obtained by mating a few no-wing females to winged males, and the stock obtained in this way was used to keep the mutant gene in existence, by breeding from heterozygous pairs.

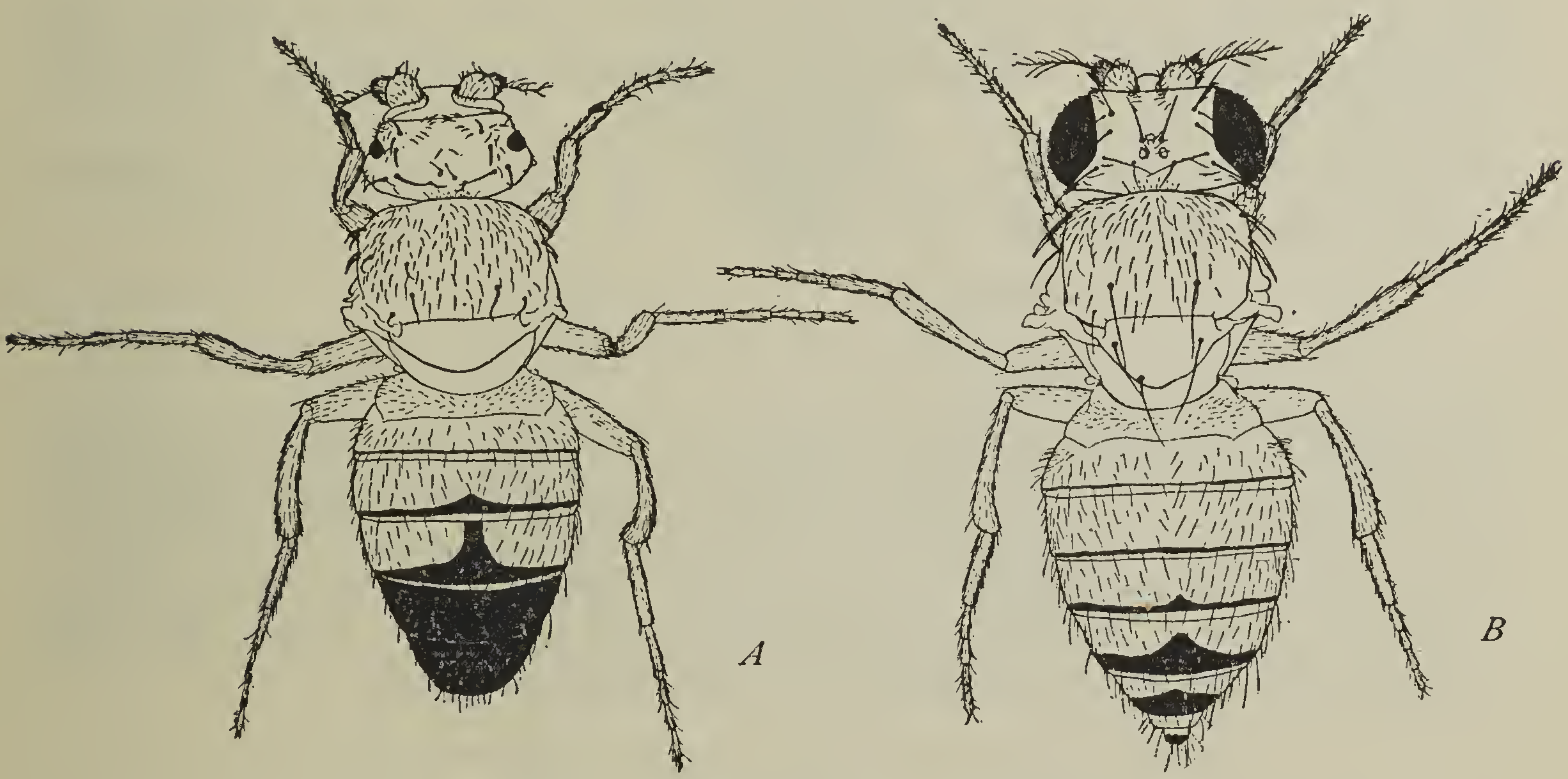


FIG. 1—No wings: *A,* male; *B,* female.

The mutant closely resembled another type called apterous that Metz has described (*Amer. Nat.* XLVIII, 1914). Apterous was also highly sterile. Its gene was located by Metz in the second chromosome. It gives no crossing over with black. At first it was inferred that no-wings was a reappearance of apterous, but it was found that its gene lies in another part of the second chromosome.

A few no-wing females were successfully mated to long-wing males heterozygous for no-wings. The expectation is equal numbers of wild-type and no-wings, but the number of flies with no-wings was far behind expectation, as the following ratios show:

Normal wings	No-wings
170	7
322	31
256	61
141	45
889	144

Long wing males and females both heterozygous for no-wings (F_1) gave the following ratios:

Normal wings	No-wings
304	14
147	2
73	2
164	7
197	4
84	4
87	21
126	33
223	7
327	19
342	15
351	24
188	43
2,613	195

The great deficit of no-wing flies is due to low viability of this mutant. The observed weakness of "no-wings" after hatching and their short life is in line with this conclusion.

Only two matings with no-wing males were successful. Bred to heterozygous long winged sisters they gave:

Normal wings	No-wings
156	22
197	19

No results were obtained when no-wings was mated to no-wings—not even in mass cultures. A few crosses between no-wing and vestigial were successful, and gave offspring not very different in appearance from vestigial. Stumps of wings were present and held vertically instead of horizontally as in vestigial, giving a fairly diagnostic difference (Fig. 2). The F_1 flies with vestigial-like wings when inbred gave the following results:

Vestigial	Vestigial-like (wings-up)	Longer-winged
35	7	1
201	77	..
130	27	..
78	19	..
54	18	..
131	32	..
157	43	..
79	23	..

The simplest explanation of this result is that no-wing is an allelomorph of vestigial, and the F_1 flies with stumps-up represent the "compound." On this assumption the expected result would be 1 vestigial to 2 compound to 1 no-wings. There must be an overlap between the first two classes if this is the explanation. Only the more striking "compounds" are separable. The absence of the no-wing class can only be explained on low viability.

This was further tested by breeding flies heterozygous for Curly and no-wings to vestigial, and inbreeding the F_1 vestigial-like flies.

The preceding crosses show that no-wings is present in the second chromosome. A balanced stock was made by crossing no-wing females to Curly males. Curly is a second-chromosome dominant, generally lethal in double dose. The Curly chromosome also prevents crossing-over in the female. Hence an F_1 Curly female bred to an F_1 Curly male will give only heterozygous Curly males and females (heterozygous for no-wings) and no-wings. The cross gave the following results:

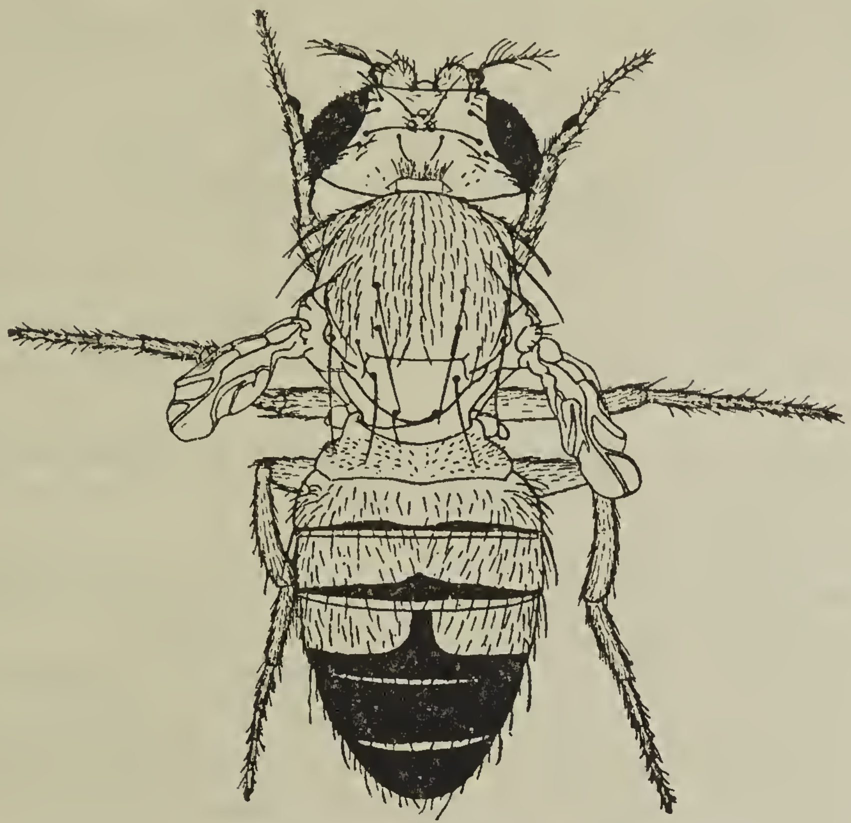


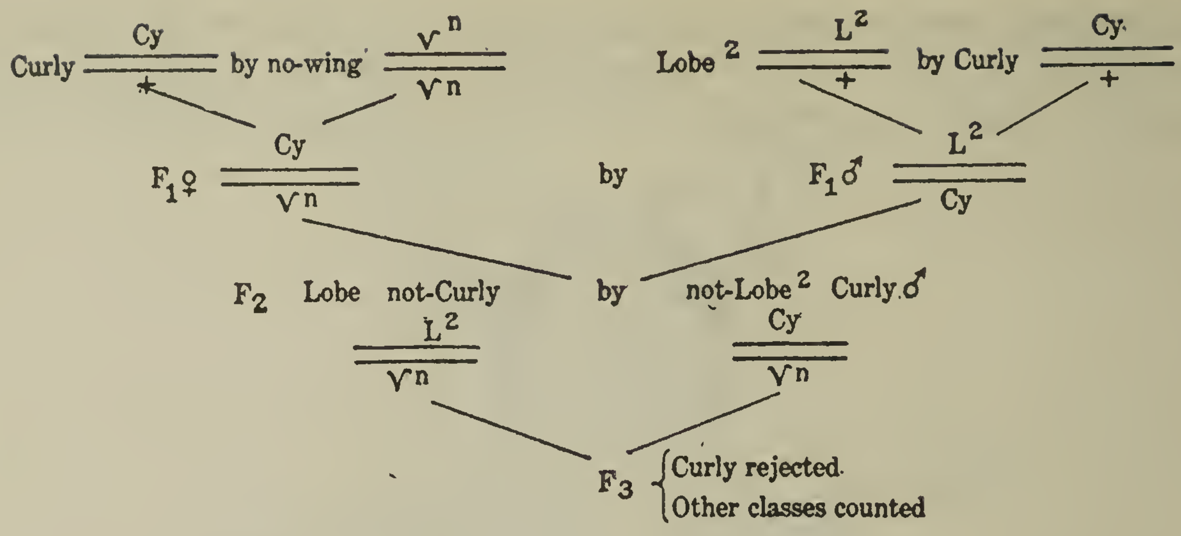
FIG. 2—Compound of vestigial and no-wings

Curly ♂ ♀	No-wings ♀	No-wings ♂
112	4	1
168	4	7
56	15	8
102	19	15
107	24	13
14	0	5
117	3	2
140	22	11
152	11	5
205	14	7
1,173	116	74

Since the homozygous dominant Curly usually dies, the expectation is 2 heterozygous Curly to 1 no-wings. The actual ratio is about 6 to 1 which again is due to the low viability of no-wings.

In one case a heterozygous Curly male was bred to a no-wings female and gave: Curly 148; no-wings 29.

An experiment had been planned to test the location of no-wings although if, as the other experiment seemed to show, it is an allelomorph of vestigial, it seemed unnecessary to carry out such a test. The different steps in the experiment are indicated in the following diagram:



(1) No-wing females were mated to Curly males in order to obtain F_1 females with Curly in one chromosome (together with the non-cross-over "factors") and no-wings (v^n) in the other.

(2) At the same time Lobe^2 was crossed to Curly in order to obtain F_1 females that had Lobe^2 in one chromosome and Curly in the other.

(3) The flies obtained in (1) and (2) were mated and from their offspring one kind of female and one kind of male were selected for mating. The F_2 females were Lobe (and not-Curly) having Lobe^2 in one chromosome and no-wings in the other. The F_2 males were Curly (and not-Lobe) having Curly in one chromosome and no-wings in the other.

(4) All the Curly offspring were rejected because they came from the Curly-bearing chromosome of the father that contained the normal dominant allelomorph of no-wings (hence concealed the crossover classes). The remaining offspring that had received the no-wing chromosome from the father (and the normal recessive allelomorph of Lobe) were separated into classes which are expected to show the crossingover in the female; the non-crossover factor having been eliminated by the selection of the not-Curly mother. The four classes obtained were as follows:

Curly wings	Straight wings			
	Normal wings		No wings	
	Lobe^2	Not Lobe^2	Not Lobe^2	Lobe^2
73	30	4	12	3
106	40	4	18	2
82	32	7	15	3
42	38	7	11	6
26	16	1	7	1
51	39	4	16	3
199	80	13	49	9
91	56	10	29	8
89	45	11	27	6
162	84	19	23	9
134	52	8	29	5
145	71	11	23	2
195	71	22	38	9
102	37	10	48	6
34	14	0	17	1
1,531	705	131	362	73

The sum of the 2 crossover classes (131 + 73 = 204) divided by the total number of flies (except Curly) *viz.,* 1,271 gives 16% crossing-over. But inasmuch as the no-wing flies are known to be very inviable the crossing-over was also calculated from the flies that had normal wings, *viz.,* Lobe[2] (705) and wild type (131). Dividing the latter (131) by the sum of both (131 + 705 = 836) gives 15.7% crossing-over. These two values are near enough together to be taken as the same.

This calculation does not show on which side of Lobe no-wing lies, but if it is an allelomorph of vestigial it is about 5 units to the left. The result places it at 15 units from Lobe[2]. This must mean that a crossover modifier was present that affected its "distance." Special tests for such a modifier were not made at the time.

WINGS-DOWN

A mutant appeared whose wings turn down and are somewhat appressed to the sides of the body (Fig. 3). The character, when fully developed, is striking, and in extreme form prevents the fly from mating or at least interferes seriously with the process. The wings drag on the surface over which the fly moves and, becoming wet, stick the fly to the food. In order to use the flies for crosses, the wings of the newly hatched fly were cut off near the base. In less extreme conditions the wings are held out and somewhat down. The linkage experiment indicates that the character may also, in a few cases, approach so nearly to the normal that it is indistinguishable from it.

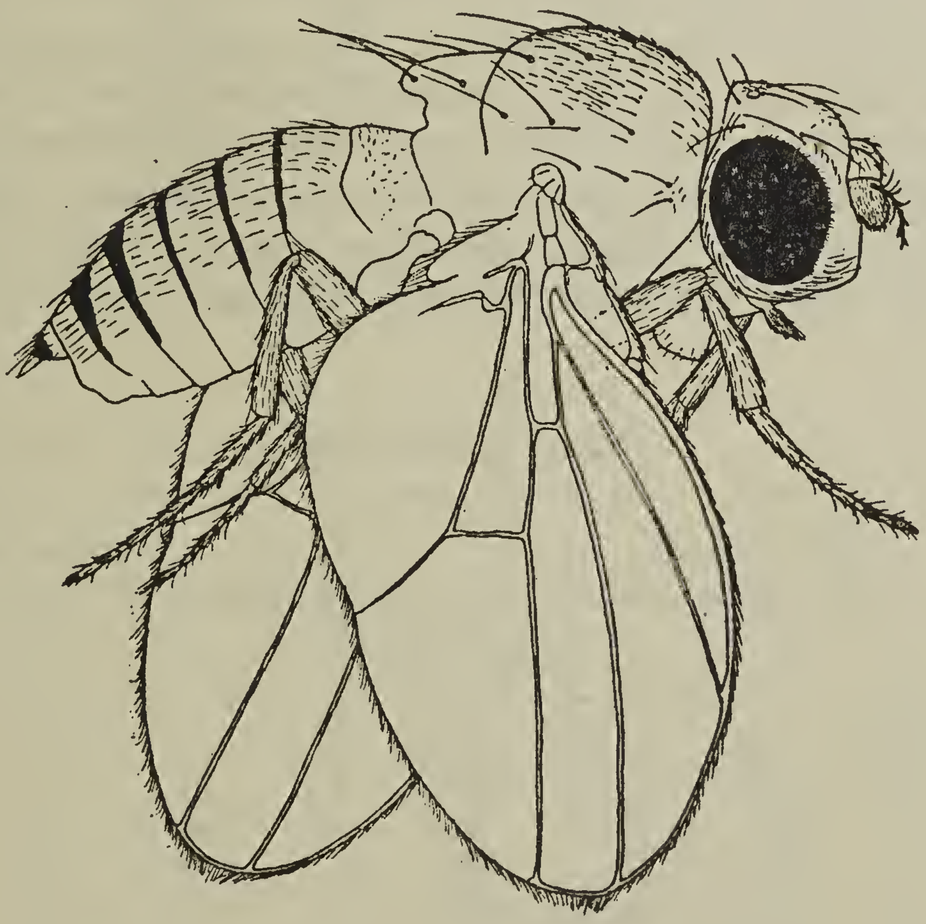


Fig. 3.

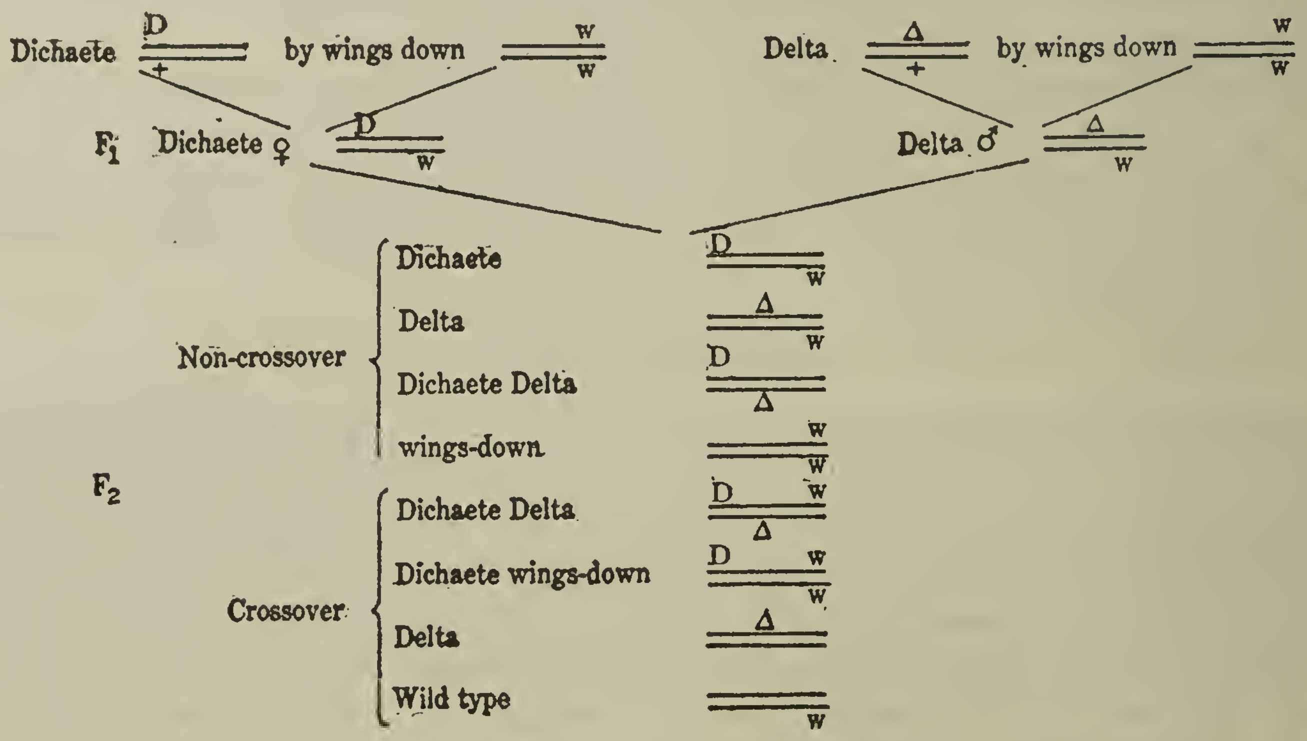
The gene was located in the third chromosome by means of the following crosses:

(1) Wings-down was crossed to the dominant Dichæte to obtain a Dichæte female heterozygous for wings-down.

(2) At the same time wings-down was also crossed to the dominant Delta to obtain a Delta male heterozygous for wings-down.

(3) Females from (1) were mated to males from (2) for the crossing-over between Dichæte and wings-down, etc.

The following diagram gives the relations of the genes involved in the experiment:



In order to make use of these F_2 data all the Delta flies are to be disregarded since only the double recessive wings-down chromosome is needed to test the crossing-over in the F_2 females. Also owing to the poor viability of wings down these too may be disregarded (although with them the results would be essentially the same). This leaves the Dichæte and the wild type flies (crossovers) as suitable material for the calculations. There were, as the next table shows, 252 Dichæte and 216 wild types. Dividing 216 by the sum of the two gives a cross-over value of 46 units.

F₁ D ♀ by F₁ Delta ♂				
Wings Straight			Wings-Down	
D	N	Δ and Δ D	D	+ (*i. e.* not-D)
64	52	83	9	1
67	47	117	12	16
13	17	40	14	9
62	54	154	16	1
46	46	163	34	24
252	216			

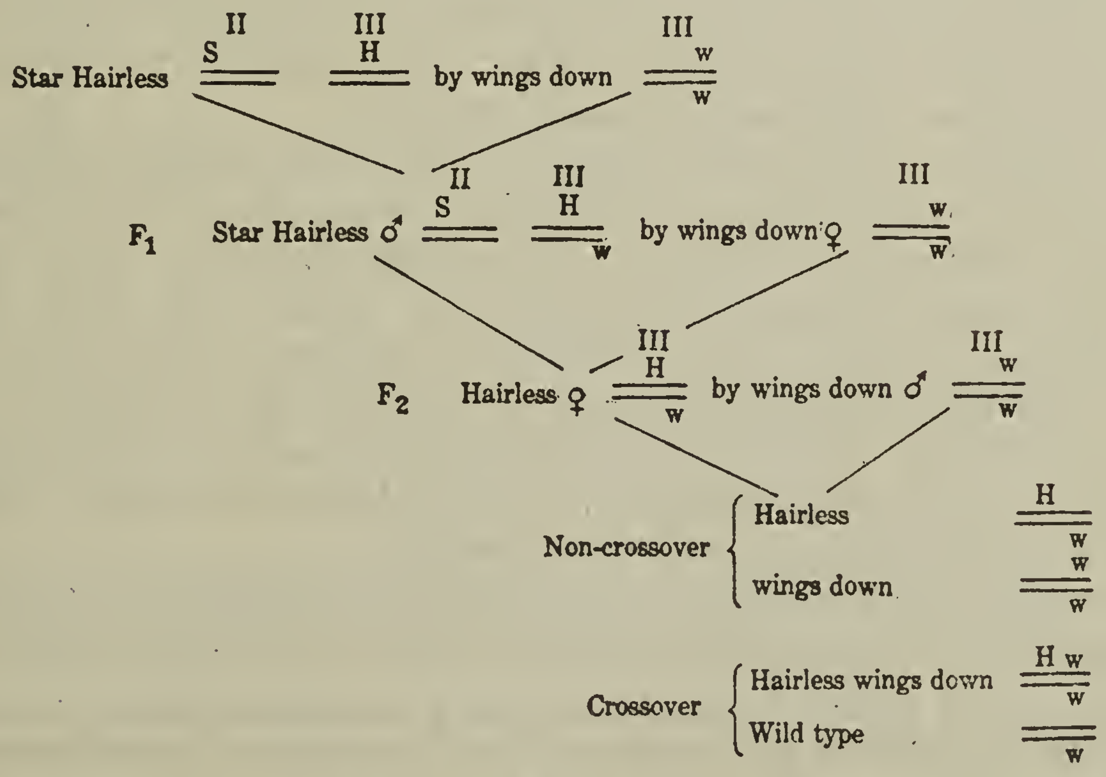
The experiment can be repeated by mating F_2 Dichæte females to F_1 Delta males. This was done with the following results:

Wings Straight			Wings-Down	
D	N	Δ and Δ D	D	N
18	7	41	0	0
10	13	29	5	0
6	4	28	0	0
2	4	30	0	0
31	17	86	3	0
67	45	214	8	0

These F_3 results give a cross over value of 40. Taking F_2 and F_3 together a value of 45 is obtained. This places wings-down far to the right or to the left of Dichæte. In order to determine on which side it lies an experiment was planned with the dominant Hairless,[1] (see next table).

Wings Straight		Wings-Down	
H	N	H	N
24	21	3	12
86	47	5	19
42	15	4	19
89	31	4	19
99	38	11	18
F_2 H ♀ 76	40	11	30
113	44	13	21
84	46	21	10
129	53	30	28
93	33	8	11
F_3 H ♀ 81	29	2	21
916	397	112	208

Advantage was taken of the fact that F_2 Hairless females when crossed to wings-down males give the cross-over sought for. The entire experiment involving the original steps with Star Hairless is indicated in the following diagram.



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[1] Star was present in the stock with Hairless, but is disregarded in the data given.

The result gives 31 units between Hairless and wings-down. Hairless is itself 29.5 units from Dichæte at 59.5. The locus of wings down is therefore at about 90 in the far right end of the third chromosome.

HIGH FEMALE RATIOS OF A SEX-LINKED CHARACTER OF *DROSOPHILA* DUE TO INVIABILITY

A pale yellowish-colored mutant, now called yellowish, appeared in the late summer of 1921 in an inbred stock in which eosin vermilion and forked were involved. The mutant appeared as an occasional male in the stock. These males were weak, and often died without mating, but finally one succeeded, and since yellowish appeared in some of the grandsons, it became probable that the character was sex-linked.

By suitable matings, yellowish females were soon obtained. When these were mated to normal males, the number of the yellowish sons was far less than that of the not-yellowish daughters. Furthermore, when a female heterozygous for yellowish was bred, the expected yellowish half of her sons fell far below that of her other class of sons. Both points are illustrated in the following cases. The progeny from yellowish females by eosin vermilion forked males (1 and 2), or yellow white males (3 and 4), gave:

	N ♀	Yellowish ♂
(1)	98	29
(2)	162	33
(3)	66	4
(4)	123	17

The deficiency in yellowish males is more striking perhaps in cultures from a female heterozygous for yellowish by a not-yellowish male. Two such counts taken together gave:[1]

N ♀ 143; $w^e vf$ ♂ 79; Yellowish ♂ 7.

The difference in the two cases, when only yellowish males are expected and when half of the males are expected to be yellowish, raises the question as to whether the result is due to competition amongst the males, or directly to the inviability of the yellowish males. This question can be better dealt with after further evidence has been presented.

When yellowish females and males are bred to each other, discrepancy in the sex ratio is not so great. From six cultures there were obtained 353 females and 231 yellowish males. The result appears to mean, since relatively more males appear, that some sort of competition takes place when normal larvæ are present that reduces the number of yellowish individuals.

When yellowish males are outbred, the sex ratio is not affected, since the sons then get their X-chromosomes from their mother. Thus six cultures out of eosin vermilion forked females by yellowish males gave 228 normal daughters and 180 eosin vermilion forked sons.

Since yellow white flies also run behind their schedules in competition, a cross was made between yellowish males and yellow white females, and the

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[1] To these may be added a few cases in which a non-virgin yellowish female that had been fertilized by a not-yellowish male gave normal daughters and yellowish sons:

N ♀ 750; Yellowish ♂ 105.

F_1 normal females were then bred to yellowish males. The results were as follows:

N ♀	Yellowish ♀	yw ♂	Yellowish ♂	N ♂
124	11	118	7	1

In the next case, an eosin female was crossed to a yellowish male, and an F_1 female was back-crossed to yellowish giving:

N ♀	Yellowish ♀	w^e ♂	Yellowish ♂	N ♂
74	0	56	1	1

In these last two cases the deficiency in the yellowish classes is very large in comparison with the yellow white and the eosin males, indicating that, even in competition with these stocks, yellowish runs behind. The two normal males are crossovers between yellowish and yellow white in one case and between yellowish and eosin in the other. They indicate that yellowish is near the locus of yellow and that of white (or of its allelomorph, eosin).

In the next experiment a yellowish male was crossed to a yellow white female and the normal daughters were crossed to yellow white males giving:

N ♀	yw ♀	yw ♂	Yellowish ♂
449	183	253	18

A closer approximation was sought by making a three point experiment by crossing yellowish males to eosin vermilion forked females and then back-crossing the F_1 female to an eosin vermilion forked male. The results from 14 cultures are given in table 1:

TABLE 1

Gray								Yellowish						
♀	+♂	w^evf♂	w^ev♂	w^e♂	w^ef♂	v♂	f♂	♀	+♂	vf♂	w^ev♂	w^ef♂	v♂	f♂
122	....	25	7	17	1	...	...	....	2	......	1	......	......	......
144	....	40	11	21	6	...	...	....	3	......	......	......	......	4
110	1	16	9	11	1	...	...	....	3	3	......	......	3	1
82	....	20	6	10	......	...	...	....	2	2	......	1	......	1
123	....	24	7	15	2	...	...	....	1	1	......	......	......	......
78	....	19	5	19	7	...	...	....	3	1	......	......	......	......
140	....	16	10	12	3	...	...	....	1	......	......	......	......	......
80	....	16	5	15	......	...	...	....	......	1	......	......	2	......
95	....	10	7	15	4	...	...	....	......	......	......	......	......	......
110	1	27	14	17	3	1	...	....	......	......	......	......	......	......
80	1	17	9	8	2	...	1	....	2	1	......	......	1	......
116	....	22	8	17	3	...	...	....	5	1	......	......	2	......
76	1	22	7	13	3	...	...	....	......	......	......	......	......	......
108	....	20	6	15	3	...	...	....	......	......	......	......	......	......
1,464	4	294	111	205	38	1	1	....	22	10	1	1	8	6

The males only are here available for cross-over data. One of the two sex chromosomes of the F_1 female carries yellowish, the other eosin vermilion forked located at 1.5, 33.0, and 56.5 respectively. The cross-overs between these three factors and those in the other chromosome are given in the fourth and fifth columns; *viz.,* (w^ev ♂ and w^e ♂). There were also four normal males. These could only arise if yellowish is to the left of eosin and a cross-over occurred between them. The reciprocal male would be yellowish eosin vermilion forked, and none were found, which can be accounted for by the scarcity of yellowish males. Among the yellowish males the non-crossover class, yellowish, is the most numerous (22). The results show that yellowish lies to the left of eosin and quite near to yellow.

Another and better crossover experiment was planned using the factors scute (sc) and apricot (w^a) that lie in this region (scute at 0. and apricot at 1.5). In the first preliminary experiment a yellowish female was mated to scute apricot males, and the F_1 normal daughters were back-crossed to yellowish. The results are given in table 2.

TABLE 2

Gray			Yellowish	
♀	sc w^a ♂	sc ♂	♀	♂
94	100	..	1	2
90	83	1	3	9
93	45	5	13	16
128	89	..	10	9
88	86	..	16	11
43	38	..	20	16
93	70	1	4	1
46	54	1	25	8
58	29	3	3	6
100	42	1	..	10
833	636	12	95	88

No crossovers between scute and yellowish were obtained, indicating that the locus of yellowish is very near scute. The twelve scute males were from crossing over between yellowish and apricot.

In the next experiments yellowish females were mated to scute apricot males, giving the F_2, backcross offspring shown in table 3.

TABLE 3

Gray			Yellowish	
♀	sc w^a ♂	sc ♂	♀	♂
93	50	2	..	15
143	80	4	..	6
142	63	2	..	7
127	46	..	..	19
120	45	2	..	9
148	52	1	..	3
108	38	..	..	3
95	28	1	..	6
84	41	..	..	15
104	53	..	..	11
93	50	..	..	7
1,257	546	12	..	101

Here also there were none of the sought for crossovers between scute and yellowish. The experiment was repeated on a still larger scale. The results are given in table 4.

TABLE 4

Gray					Yellowish	
♀	sc w^a ♂	sc ♂	w^a ♂	+ ♂	♀	♂
250	93	8	..	..	..	..
201	104	4	..	..	..	..
253	76	2	..	..	2	..
151	69	2	..	..	..	..
269	71	1	..	..	..	..
152	73	1	..	..	1	..
153	59	2	..	..	1	..
58	22	1	..	..	..	..
225	91	1	..	..	..	..
222	88	4	..	..	..	..
227	109	1	..	1	..	1
263	107	1	..	..	..	2
199	78	2	..	..	..	1
123	32	4	..	..	..	..
290	142	4	..	..	..	..
156	68	2	..	..	..	1
358	92	1	..	..	..	2
43	16	..	..	..	..	..
183	76	..	..	1	..	..
104	41	..	..	..	..	..
200	78	2	..	..	..	1
166	76	2	..	..	1	1
231	189	2	1	..	..	..
287	112	1	..	..	..	3
190	81	..	2	..	..	1
160	50	1	..	..	..	..
266	105	..	..	..	..	3
205	81	1	1	..	..	..
100	90	5	..	..	1	1
182	71	2	..	..	..	..
208	99	2	..	..	..	2
235	70	2	..	..	..	..
119	45	4	..	..	..	..
90	42	5	..	2	..	1
451	135	2	..	..	..	2
282	113	5	..	..	..	..
216	147	4	..	..	..	..
7,468	3,091	81	4	4	6	22

There are several alternative possibilities in respect to the occurrence of the normal males in the last experiment. First, contamination is always a possibility, but there was no special reason to suspect it in these cases. Second, the character scute is variable, and may possibly, at its extreme, overlap normal. Realizing this difficulty, two of the four normal males were tested by breeding them to scute. They gave wild type daughters, hence were wild (standard) flies. Unfortunately, the other two were either not tested or left no offspring, but that some of them may have been slight scute, was shown by another test that was made. Eight apricot daughters appeared not to be scute (*i. e.*, they were normal) but three gave scute daughters which showed that they were really scute. There was then a good chance of mistaking an extreme fluctuant of scute for normal flies.[1]

Third, mutation of scute to normal might be a possible explanation, and this, if it happens, would account for the apricots, but not for the wild type males. Fourth, mutation of yellowish back to gray (wild type) would account for the wild type male. This was tested on a large scale by breeding pure stock. No reversion occurs, hence this hypothesis fails unless it be supposed that such reversion occurs more frequently in pure stock, *i. e.*, it is more frequent when both yellowish and its normal allelomorph are present.

The largest class of males is the sc w^a or non-crossover class; the next largest is the single crossover scute between scute and apricot.

The critical classes that test the location of yellowish are the double crossovers. If yellowish is to the right of scute, the double crossovers (which are not possible within this distance (1.5 units)) would be normal and scute yellowish apricot. A few normal males were, in fact, found. The absence of scute yellowish apricot might be explained as due to the non-viability of yellowish. But, as stated, double crossing-over has never been found in a distance of 1.5. On the other hand, if yellowish lies to the left of scute, normals may result as single crossovers, and the corresponding class of scute apricot yellowish might be absent because of the non-viability of yellowish. The double crossovers would then be apricot and yellowish scute. The former was present, and the absence of the latter could be explained as due to inviability. But the double apricot appears as often as the single crossover, wild type, which can not be the case. The most probable explanation is that the flies, classified as apricot, were not crossovers at all, but scute apricot flies in which scute was suppressed. This interpretation places yellowish at the left of scute. In this respect the results would agree with those of Mohr, published later (1923). At this time Mohr described (*Studia Mendeliana, Brunæ*) a mutant that he called chlorotic. It shows the same characteristics that I had found for yellowish. Its locus was given at 0.1 to the left of yellow. My own results are consistent with this location for yellowish if the scute class be interpreted as due to overlap. Therefore, it is almost certain that chlorotic and yellowish are the same, or else allelomorphs.

Yellowish Males by Double Yellow Females

The discovery of the double yellow female (a female with two united X-chromosomes each carrying the recessive gene for yellow) offered another opportunity of studying the viability of yellowish, and also furnished a simple means for carrying on the race. Inasmuch as half the zygotes die when any male is bred to double yellow, the population is kept down and if competition explains the deficiency of the yellowish males there is a chance here to get evidence of it. Furthermore, since the yellow individuals themselves are less "vigorous" than grays as a rule, this again might give the yellowish male a better chance. The results are given in table 5.

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[1] A repetition of the experiment gave the following results:

Gray: ♀, 2,349; sc w^a ♂, 966; sc ♂, 22; w^a ♂, 12; N ♂, 3. Yellowish: ♀, 0; ♂, 10. Here again twelve not-scute apricot males appeared. Three of these were bred to a YY female and gave all scute sons. Therefore these three males were really scute though they looked like normal. Payne (1920) has described a modifier that changes scute to normal. There were also three apparently normal (*i. e.*, not-scute) males in this back-cross. Bred to scute they gave scute daughters showing that genetically they were scute.

TABLE 5

Double yellow ♀ × yellowish ♂			
yy ♀	yellowish ♂	XXX ♀	XX ♀
109	9	..	..
233	68	5	..
125	23	4	2
112	5	..	1
57	8	2	..
133	19	2	..
172	43	2	2
251	80	18	1
64	10	8	..
1,256	265	41	6

The number of yellowish males is perhaps proportionately greater than in cases where the females are wild type, which may be due to either alternative offered above, or may be due to the different autosomal group received from the double yellow stock.

In this experiment there were a few gray females, as expected. Most of these have three X's as is obvious from their somatic peculiarities. It has been shown by Lilian V. Morgan that this kind of exceptional female has a double (yellow bearing) X and a third single (gray bearing) X-chromosome. Those I have obtained were found to be sterile, which is a known peculiarity of this kind of female. In addition, a few normal XX gray females appeared. They are expected whenever the double X's break apart and an egg has one X left in it. Such an egg fertilized by an X (gray bearing or female producing sperm) is expected to give a normal female. An XXY, non-disjunctional female, would also be the same phenotype. Unless these females are tested, however, one can not always be certain that they are not XXX females that sometime approach very nearly XX females in their structure. If they produce offspring they are XX females, if not they are XXX's, since many tests of XXX's, that are obviously such, have shown that they are sterile.

GYNANDROMORPH

In a cross of yellow white females by yellowish males, a yellow white male appeared with a red rim on the upper edge of each eye forming a crescent of red ommatidia. Since the crescents of the two eyes were symmetrical, the fly looked like a new mutant. Crossed to yellow white sisters he gave 32 yellow white females and 35 yellow white males. Crossed to yellow white females (of another stock) he gave 187 female and male offspring. In later generations no red eyed flies appeared.

It is clear that the fly, if a mutant, was only a somatic one, and the change did not involve the germ-cells; but it is much more probable that it was a gynander in which the red color of the crescent is due to an XX condition of that part—one X carrying yellow white, the other yellowish. The rest of the fly was male with spermatozoa in the testis. As he was fertile, he was an XY and not an XO male, because the latter kind of male is sterile. Therefore, the case is not a simple X elimination female case but arose either from an XY egg fertilized by an X sperm followed by elimination, or from an XY primary non-disjunctional sperm followed by elimination; or from a two-nucleated egg one X, one Y (reduced nucleus) fertilized by two X-sperms.

A MUTANT ALLELOMORPH OF TRUNCATE CALLED DUMPY

A mutant appeared in one experiment with yellow prune that had the following characteristics: the hairs on the thorax are arranged in whorls or pits resembling in this respect the mutant vortex. The wings are obliquely truncated and about two-thirds full length. The general appearance of the fly, as its name suggests, is short and thick.

A yellow prune dumpy male was crossed to a wild female. The F_1 flies were all wild type. These inbred gave the following data:

Normal		Dumpy		Yellow prune Dumpy	Yellow prune
♀	♂	♀	♂	♂	♂
49	38	20	17	17	25
51	43	12	12	8	56
85	36	20	18	13	25
90	51	29	25	7	34
76	30	9	7	7	33
24	14	4	1	2	6
375	212	94	80	54	179

Since there were dumpy females in F_2 it is obvious that the dumpy gene is not in the X chromosome. There were 766 not-dumpy and 228 dumpy F_2 flies, approximately a 3:1 ratio.

The reciprocal cross gave, in F_1, wild type females and yellow prune males. These inbred gave:

Normal		Dumpy		Yel. Prune		Yel. Prune Dumpy	
♀	♂	♀	♂	♀	♂	♀	♂
58	58	22	25	41	40	5	11
25	22	27	16	28	18	12	15
83	80	49	41	69	58	17	26

Another sex-linked character miniature was also used with dumpy. Miniature males by dumpy females gave F_1 wild type males and females. These inbred gave:

Normal ♂ ♀	Dumpy ♂ ♀	Miniature ♂	Miniature Dumpy ♂
207 212	83 83	62 66	7 19
419	166	128	26

The results are here the same as in the last cross.

Dumpy was crossed to vestigial giving wild type males and females. These inbred gave:

Normal	Dumpy	Vestigial
215	63	8
150	47	46
61	29	24
145	37	38
36	33	10
607	209	126

The F_1 result shows that dumpy is not an allelomorph of vestigial. The absence of the double recessive, if not due to inviability, indicates that dumpy is in the second chromosome. No crossing over in the male will explain the absence of dumpy vestigial, for the expectation then is two wild type flies to one dumpy to one vestigial, which is the realized result with some allowance for inviability of vestigial.

In order to get direct information as to chromosomal location dumpy was crossed to Star Dichæte females and the F_1 Star Dichæte males were then back-crossed to dumpy females with the results given below. Only the Star and the dumpy flies were classified. Since all the Stars are not-dumpy and all the dumpy are not-Star, the gene must lie in the second chromosome.

Star (not-Dumpy)	Dumpy (not-Star)
54	43
57	52
26	19
19	23
27	30
34	39
8	4
44	38
269	248

At the same time that the test was under way, a similar cross was made with the third chromosome character Delta (Δ). Dumpy was crossed to Delta and the F_1 Delta females were crossed to dumpy males. The offspring are given below. Equality in the four classes resulting was expected, and was found. The result shows that dumpy is not in the third chromosome.

Normal ♂ ♀	Dumpy ♂ ♀	Delta ♂ ♀	Delta Dumpy ♂ ♀
23	16	11	19
9	3	9	13
20	20	18	21
37	45	37	21
37	45	37	35
49	29	34	26
24	7	10	8
53	51	54	58
28	36	38	45
22	24	28	28
23	18	26	20
12	11	7	2
26	18	16	30
18	15	10	14
44	18	39	17
45	41	47	33
470	397	421	390

In order to locate dumpy within the second chromosome it was crossed to Lobe and the F_1 heterozygous Lobe female was bred to dumpy male (stock). The results were as follows:

Normal	Lobe	Dumpy	Lobe Dumpy
63	76	102	59
30	30	60	19
95	111	113	58
97	137	140	102
71	48	62	44
356	402	477	282

The data give a crossover value of 41.3 units from Lobe. Lobe lies at 72. If the locus is to the left of Lobe it lies more than 41 units from Lobe and on account of double crossing over roughly about 20.

Dumpy was next crossed to Truncate that lies at locus 13.0 in the second chromosome. Half the offspring were dumpy-like and half normal, which proves that dumpy is an allelomorph of Truncate. Truncate is a dominant that is lethal when homozygous. Each Truncate fly is heterozygous for normal, hence, when bred to dumpy the Truncate-dumpy compound constitutes half of the offspring (resembling dumpy) and half will be normal in appearance (heterozygous for dumpy) since dumpy is recessive. Thus:

$$\text{Truncate}\ \frac{T}{+}\ \text{by dumpy}\ \frac{d}{d}$$

$$\frac{T}{d}\ \text{and}\ \frac{+}{d}$$

While Truncate is dominant and lethal in double dose its allelomorph dumpy is recessive and not lethal.

VII

EXPERIMENTS WITH DROSOPHILA

By T. H. Morgan

With five text-figures

EXPERIMENTS WITH DROSOPHILA

By T. H. Morgan

INTRODUCTORY

Under this general title I have brought together some scattered observations and experiments on *Drosophila melanogaster*, most of them carried out several years ago. They have only a belated interest at present, but since they contain a certain amount of data not put on record (occasional references have been made to some of them in the Carnegie Institution of Washington reports), it seemed worth while to publish the result.

HYBRID SEX RATIOS OF *DROSOPHILA MELANOGASTER* BY *D. SIMULANS*

In March 1909, L. S. Quackenbush observed some extraordinary sex ratios from pair-matings of *Drosophila*. The stock came originally from wild flies brought from Woods Hole, Massachusetts. The flies had probably been collected the preceding summer. They were supposed to be *Drosophila melanogaster* (then called *ampelophila*), but as the sequel shows, there must have been also present *Drosophila simulans*. These two species are so much alike that they require an expert to separate them.

Out of a large number of matings that gave normal results, Quackenbush found that ten pairs gave only female offspring (respectively 108, 104, 73, 63, 45, 43, 33, 31, 68, 30); in addition, one pair gave 1 male and 52 females. The reverse situation appeared in another mating in which 152 sons and no daughters appeared. During the following summer and winter, many attempts to get further results of the kind failed.

We realize now that these extraordinary sex ratios were due to the presence, in the "Woods Hole *melanogaster*" stock, of some *D. simulans* individuals. Not only do the sex ratios indicate this, but also the fact that all the females in unisexual female broods and all the males in unisexual male broods were sterile. At the present time, we can obtain similar results at will by cross-mating the two species; for, if they are crossed *melanogaster* ♀ by *simulans* ♂ they give high or exclusively female ratios, and, reciprocally, high or exclusively male ratios.

Nine years later (1918) A. M. Brown, also working in the Columbia University Zoological laboratory on the problem of percentage of crossing-over when different wild races of *Drosophila melanogaster* are used, found that when the males of a certain stock, supposedly *D. melanogaster*, brought in by A. H. Sturtevant from Kushla, Alabama, were mated to females of other stocks of *melanogaster* only females were produced. No offspring were obtained from the reciprocal crosses.

During the spring and summer of 1919, it became evident from observations of Bridges and of Sturtevant that there are constant structural differences between the Kushla stock and other stocks in the laboratory, and that the Kushla stock is a new species of *Drosophila*, one so similar to *melano-*

gaster that it had never been recognized before as a different species. Sturtevant (1921) has named it *D. simulans.*

Before it was known that this difference between the races is "specific" I began to use some *simulans* stock (that had been sent to us by C. W. Metz) in order to see whether by feeding one of the races (then so-called) on the crushed bodies and juices of the other, the sterility between the races when, as then supposed, the cross was made one way, could be overcome.

As was to be expected, no positive results were obtained even after feeding the larvæ of the new "race" for three generations on banana mixed with the juices of the other race; but I found in this way that occasionally a cross between *simulans* female and *melanogaster* male gave offspring, all of which(in many cases) were males.[1]

On July 18, 1919, three virgin (*simulans*) females were mated to several "California" (*melanogaster*) males. The line from which these females came had been fed for three generations on mashed *melanogaster* mixed with ripe banana. There were 107 sons produced and no daughters. These sons, it now seems, may have come from only one of the females. On July 5, four virgin (*simulans*) females were mated to "California" (*melanogaster*) males and gave 74 gray males and 55 yellow males. These males may have come from one female heterozygous for yellow, or possibly a yellow female may have been amongst the four mothers used since at this time both kinds of females were present in the stock of *simulans*. The old females from the last culture were isolated and produced a second lot of 39 males.

This cross was continued at intervals for two years. It is so difficult to get successful matings that at least several hundred attempts were made, sometimes in pair matings and at other times in small mass-cultures. The latter give better results, but since the females of *simulans* are sometimes fertilized a few hours after emerging there is always the risk that one such female may be present. The outcome of experiments in 1919 (October and November) were as follows:

A. One yellow *Drosophila simulans* female by *melanogaster* male (California wild stock) gave 9 males. These were sterile with California females.

B. One (or more) yellow *Drosophila simulans* female by California male gave 80 yellow males and 31 gray females. This is the largest number of females that I have obtained from this cross. The occasional appearance of females in these crosses will be discussed below.

C. A similar cross gave 44 yellow males and no females.

D. The same combination as above gave 80 yellow males and 5 gray females.

E. A similar cross gave 27 yellow males and 2 gray males (fig. 1). It may appear that the two gray males that are unexpected could be explained as due to non-disjunction. For example; if in this case, an egg that had lost both its yellow-producing X-chromosomes had been fertilized by a female-producing sperm of *Drosophila melanogaster,* a male would be expected that would be gray in color. Such a result would be significant, because it would show that the X-chromosomes of *melanogaster* plus the rest of the haploid group of that species when added to the haploid group of *simulans* (less the X-chromosome) produces a gray male that is a hybrid. His single X would be different from that of the other yellow hybrids, each of which got its X from its mother. If, however, they arose in this way they would be XO males, and, therefore, sterile (whether hybrid or not). The two gray males were tested with *melanogaster* and found to be sterile. This test fails, however, to determine whether they are XO males or hybrids which would also be sterile. On the other hand Sturtevant found later that this combination of an X from *melanogaster* plus a set of autosomes from each species is inviable. There is another possibility in connection with the interpretation of these males that will be considered later.

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[1] This result was referred to in 1920 (Morgan. Am. Nat. L, pp. 385-410).

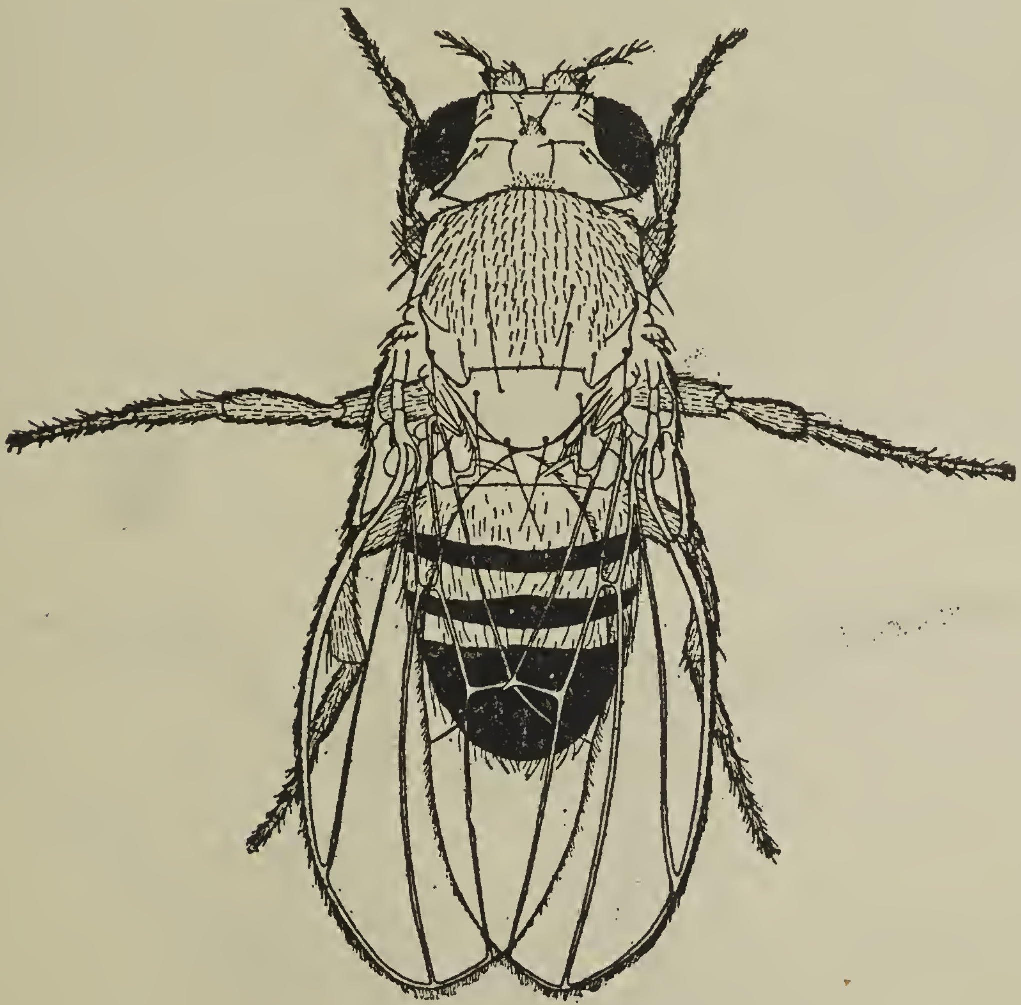

FIG. 1

The occurrence of "additional" females in two of the above crosses calls for further attention. That these females are hybrids also is shown by the paternal gray color and by their sterility. Their occasional presence shows that the diploid complex (with a set of autosomes and an X from each species) in *simulans* protoplasm can sometimes come through, although it does not do so as a rule. These and other results described below show moreover that certain combinations produce females more often than do others. Sturtevant has later traced this to particular strains. It should be observed in this connection that these "occasional females" have the same genetic composition as have the females that appear in the reciprocal cross, but the egg protoplasm is different; in one case it is pure *simulans* and in the other pure *melanogaster*. Since one kind of hybrid female is rare and the other hybrid female is common Sturtevant (1920) concluded that this diploid complex is much more likely to develop in a *melanogaster* protoplasm than in a *simulans* protoplasm. The former females are large and active, but are sterile. The latter are also sterile.

In August 1919, several reciprocal crosses were made (*melanogaster* female by *simulans* male). They yielded the same results that Brown had recorded. In nine cultures that "went" the following number of daughters (only) were obtained, *viz.*, 95, 86, 138, 66, 77, 22, 65, 40, 90.

A large number of other crosses (about 60) were carried out with *simulans* females and *melanogaster* males from different sources in the hope of finding a more fertile stock for crossing. Not one successful cross occurred in the entire lot. This will serve to show how difficult it is to carry out this mating. Later results by Sturtevant have shown that the choice of the female rather than of the male is the more important factor in success, for the result depends upon whether the mating takes place rather than on the development after mating. It was a fortunate circumstance that led me in the earlier experiments to use a weak stock (yellow) for the mother of many of the crosses. This relation may explain why Brown did not get offspring from this combination between wild stocks.

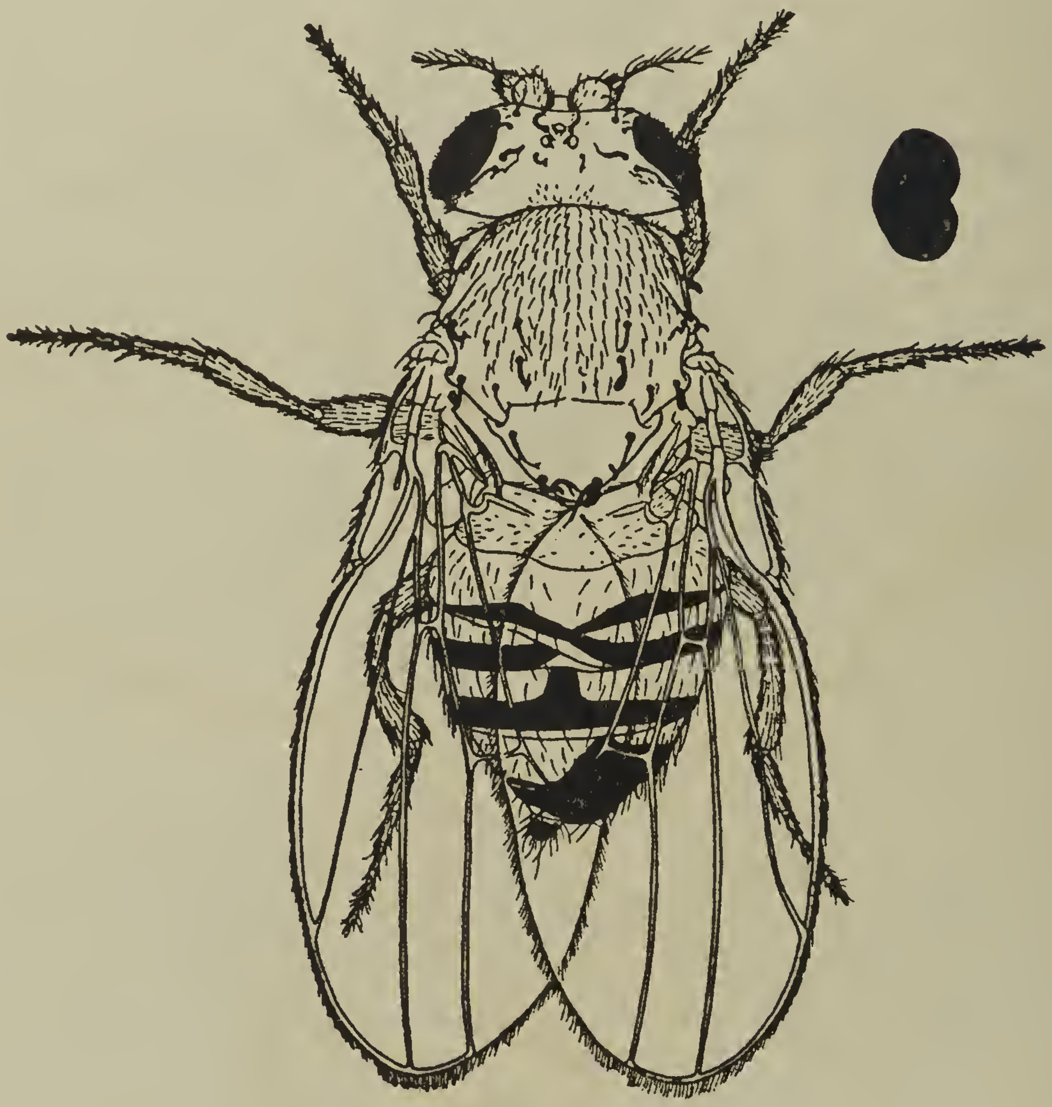

Fig. 2

During the winter and spring of 1920 some further crosses between yellow *simulans* females and dominant mutant stock of *melanogaster* were made. Yellow forked *simulans* females by *melanogaster* Lobe males gave 145 males not-lobed and 14 gray females also not-lobed with normal setæ. The result shows that Lobe is not a dominant in the cross although it is an intermediate dominant within *melanogaster* stock. In the hybrid the character is not even intermediate, or, to put it differently, wild type is dominant.

In another cross, a gray forked *simulans* female (heterozygous for yellow) was crossed to Lobe *melanogaster* male. There were produced 43 yellow forked males and 58 gray forked males. None of these 101 males showed the Lobe character.

Bar eye is one of the best dominant mutants of *Drosophila melanogaster*. When Bar males were crossed to yellow forked *simulans* females the following offspring were obtained in eight cases:

	1	2	3	4	5	6	7	8
Yellow forked males...	50	2	23	41	64	64	6	46
Bar gray females......	0	0	3	2	1	1	1	0

The heterozygous Bar eye of these hybrid females is different from that in the normal heterozygous Bar eye, being much broader, *i. e.*, more rounded. In other words, Bar is less dominant in the hybrid. The eyes of two of the hybrid Bar females are shown in figures 2 and 3. Figure 2, a hybrid, has wide Bar eyes as best seen in face view (above to the right). In figure 3, the right eye is very wide Bar while the left eye is entirely round. Both of these females had rudimentary ovaries, and in both, the rows of hairs on the thorax have an irregular distribution—another common feature of these weak female hybrids. In these two figures the abdomen is irregularly banded, but this is not a constant feature.

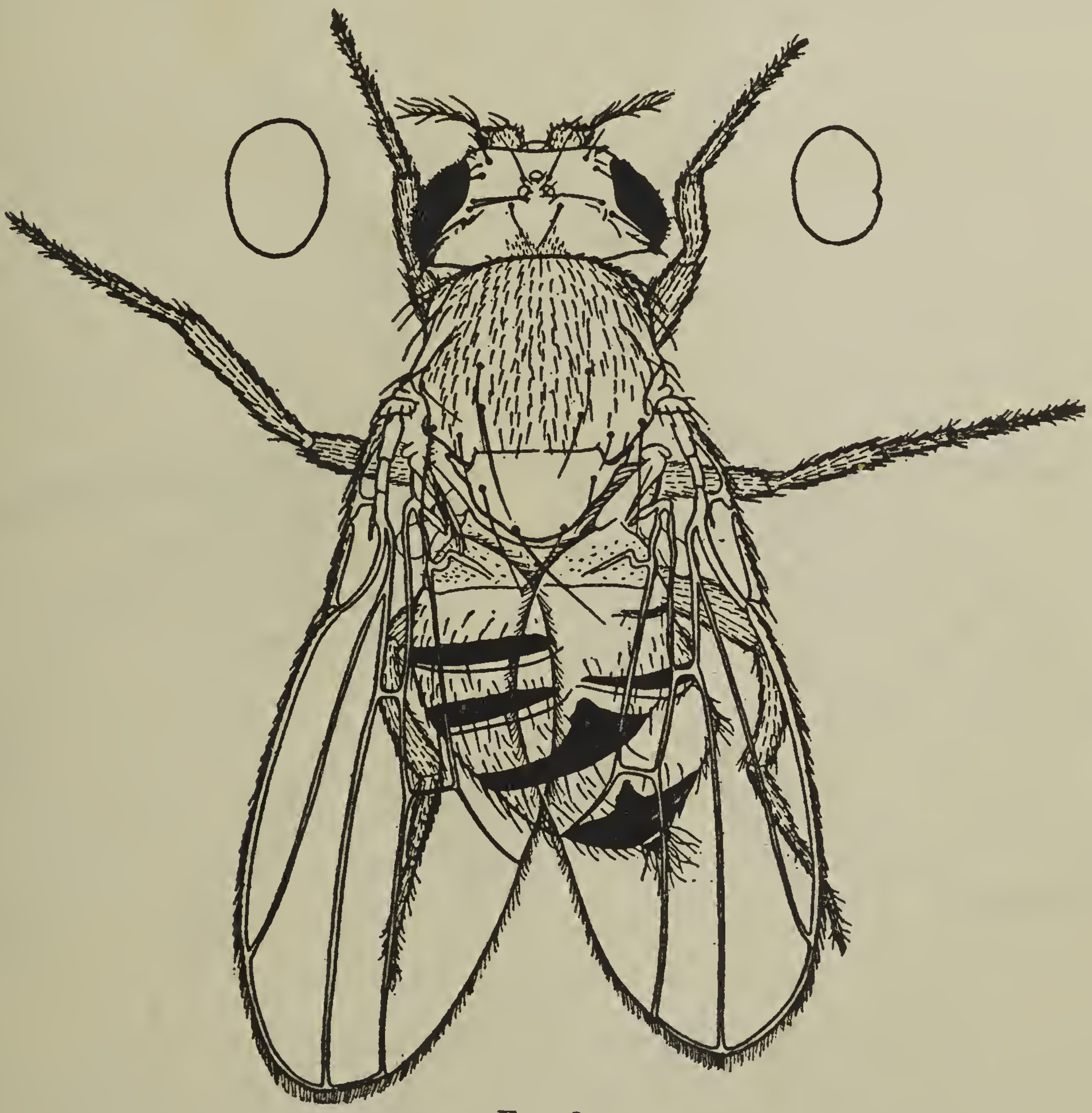

FIG. 3

A cross was made to Star, which is another *melanogaster* dominant character carried in a heterozygous condition. Half of the offspring are expected
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to show the Star character. In the present instance the gray forked *simulans* female was heterozygous for yellow; some of the sons are gray and others are yellow. Thus:

Gray forked female *simulans* by Star male *melanogaster*.

Males			
Star		Not-star	
gray forked	yellow forked	gray forked	yellow forked
70	24	125	52

In addition there were two gray, not-star females. Since about half the males are Star, it follows that Star is dominant in the hybrid.

In another experiment Star Dichæte *melanogaster* males were crossed to yellow forked *simulans* females. Amongst the male offspring there were some that were yellow forked and had Dichæte wings (fig. 4). Dichæte is therefore dominant in the hybrid.

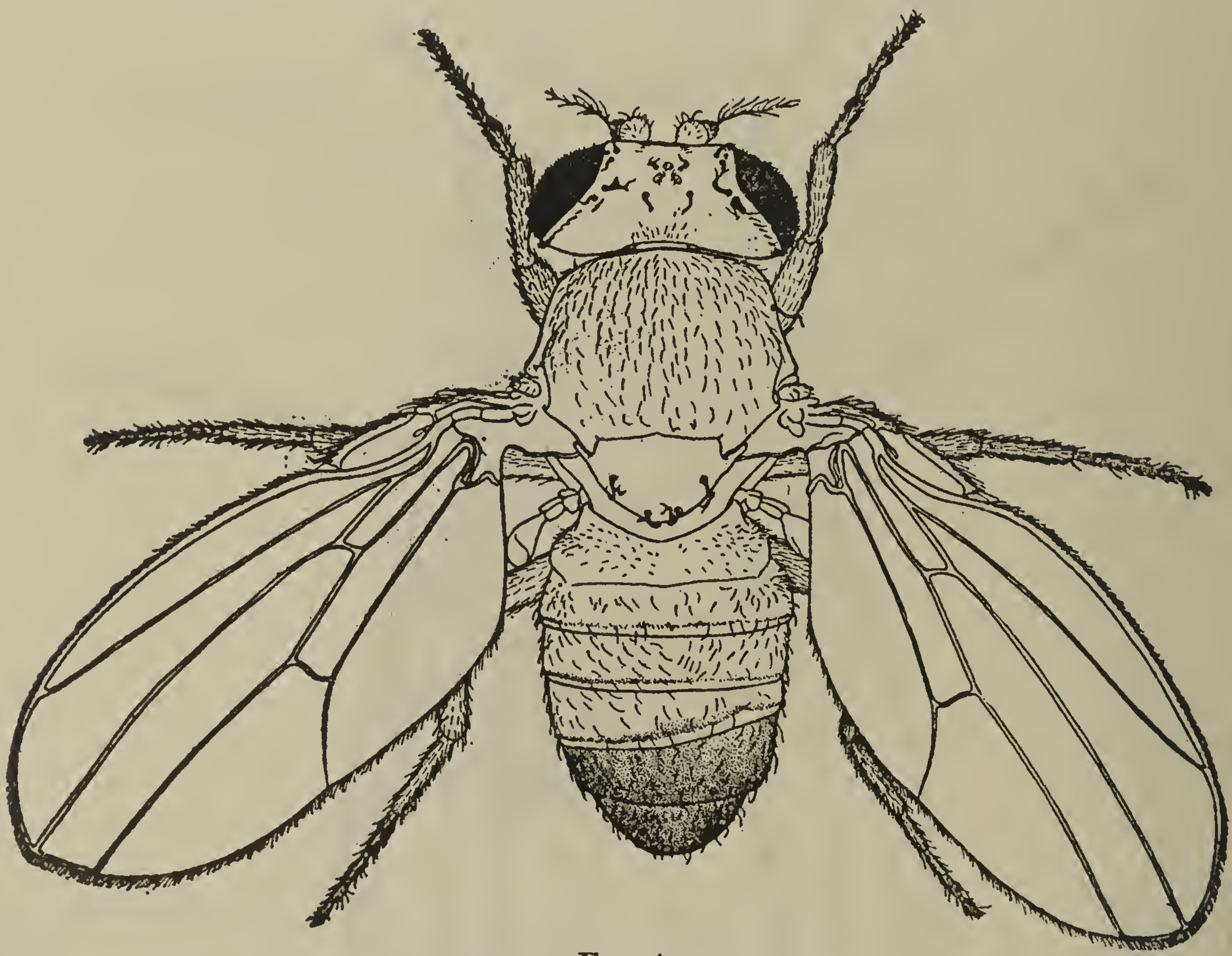

Fig. 4

Finally, a very great number of attempts were made to cross a mutant dachs-like type that had appeared in my cultures with the similar looking male of *melanogaster*. Probably 500 "dachs" *simulans* females were mated to *melanogaster* dachs males, and in not a single case were offspring obtained, probably because no successful mating took place. It should be stated that nearly all of these *simulans* dachs females were gray, and these gray flies are more vigorous than yellow dachs that had been used in most of the earlier experiments.

The reciprocal cross was also obtained. Five dachs virgin *melanogaster* females were mated to 16 *simulans* males. All (71) the offspring were female and had normal legs. Their bristle characters clearly showed that they were hybrids. It appears, therefore, that the "dachs" mutation in *simulans* is different from that in *melanogaster*. Sturtevant (1921) has obtained the same result by a slightly different procedure. He crossed *simulans* dachs male to not-dachs (whose chromosome III was marked by another mutant gene). The F_1 female was backcrossed to a dachs male. The results showed that *simulans* dachs is to the left of scarlet in chromosome III, while *melanogaster* dachs is in chromosome II. Sturtevant also obtained wild type hybrids that had two dachs genes, one from each parent (Proc. Nat. Acad. VII, 1921).

In addition to dachs, two other new mutants appeared in my cultures of *Drosophila simulans*. One, a male, had wings like those of our miniature stock. It died without offspring. Another mutant present in the yellow *simulans* stock had spread-out wings. Its relation to similar mutants in *Drosophila melanogaster* was not made out.

The Hybrid Sex Ratio

The genetic evidence leaves practically no doubt as to the cause of the high female ratio when the cross is made one way and the high male ratio when it is made in the opposite way. In both, one class of individuals is deficient because most individuals fail to come through. Thus, when yellow forked *simulans* female is mated to wild *melanogaster* male all the female offspring die in most cases, but occasionally, and from special stocks, a few females emerge. In such a case the sex-linked characters of the sons (yellow forked) show that they received their single sex chromosome from their mother, while the gray winged and not-forked daughters must get these characters from the X-chromosome of the father. This, and other similar evidence, shows that the high sex ratios are not due to "changing the sex" of the individual, as has been claimed in the case of other hybrids, but to the survival of one kind of individual only or largely, in this case the male. The occasional appearance of females in this cross is interesting, as indicating that at times the development of female individuals may be carried to the point where one or more may emerge.

In this connection it is interesting to find that the hybrid females from the reciprocal crosses are viable although sterile. The two kinds of females are identical as far as their chromosomal complex is concerned since each has one set of *simulans* chromosomes and one set of *melanogaster* chromosomes. The differences in the result must be ascribed to the chromosomes acting through the egg cytoplasm of *simulans* in one case and of *melanogaster* in the other, as Sturtevant pointed out. If, however, the development of the females, in the combination *simulans* female by *melanogaster* male, continues into late larval life and occasionally into the adult, it is difficult to understand why the egg cytoplasm may not have been changed, by the time the larval stages are passed through, into the same condition as that of the reciprocal cross. Sturtevant (1920) writes:

> "Analysis of these results indicates that the egg cytoplasm must play a part in determining what types survive. We may represent this cytoplasm by the symbol

C, and denote the origin of it and of the chromosomes by sub-letters (m for *melanogaster,* s for *simulans*). The types investigated may then be tabulated as follows:

♀	♂
$C_mX_mX_s$,—lives	$C_mX_mY_s$,—dies
$C_mX_mX_sY_m$,—lives	$C_mX_mY_mY_s$,—dies
$C_mX_mX_mY_s$,—dies	$C_mX_sY_m$,—lives
$C_sX_mX_s$,—usually dies	$C_sX_sY_m$,—lives
$C_sX_mX_sY_s$,—usually dies	
$C_sX_sX_sY_m$,—lives	

"The Y would not be expected to affect the result, and may probably be disregarded. The above formulae then simplify as follows:

♀	♂
$C_mX_mX_s$,—lives	C_mX_m,—dies
$C_mX_mX_m$,—dies	C_mX_s,—lives
$C_sX_mX_s$,—usually dies	C_sX_s,—lives
$C_sX_sX_s$,—lives	

"This table presents many anomalous points. The difference between the two kinds of regular females ($C_m X_mX_s$ and $C_s X_mX_s$) can only be due to a difference in the cytoplasm of the mother, since the chromosomes of these two types must be identical.

That hybrid females should develop in *melanogaster* cytoplasm only in case they carry a *simulans* X is surprising. This result must be due in part to the autosomes (or to the *simulans* Y), since $C_m X_mX_m$, that dies as a hybrid, is the formula of the normal *melanogaster* female. In general, it appears that hybrids develop only if they carry a *simulans* X, but that in the presence of *simulans* cytoplasm a *melanogaster* X usually inhibits development even though a *simulans* X is also present."

Sturtevant's table (above), that records the kind of Y-chromosome present, might be interpreted to mean that the presence of the *simulans* Y (Y_s) with the hybrid composition kills the fly. Thus $C_mX_mX_mY_s$ dies. This fly has the normal *melanogaster* cytoplasm and X-chromosomes but has hybrid autosomes and a *simulans* Y-chromosome. Again the male, $C_mX_mY_s$, dies. He has the normal composition for *melanogaster* cytoplasm and X-chromosome, but has hybrid autosomes and a *simulans* Y-chromosome. Similarly for $C_mX_mY_mY_s$, which is not saved by the Y_m chromosome.

On the other hand, the table indicates that the presence of the *melanogaster* Y-chromosome under hybrid conditions does not have such a deleterious effect.

The two exceptional males (page 205) seem to offer a test case. Their formulæ would be C_sX_m plus the hybrid autosomes. They should have no Y-chromosome. If there is no error in this interpretation of the character or origin of these two exceptional males, their survival is favorable to the suggestion made above that it is the *simulans* Y in *melanogaster* cytoplasm, plus a hybrid autosomal condition that causes the death of the missing classes.

BIBLIOGRAPHY

MORGAN, T. H. 1919. Study of the constitution of the germ-plasm in relation to heredity. Carnegie Inst. Wash. Year Book No. 18, 324–325.

———, 1920. Variations in the secondary sexual characters of the fiddler crab. Amer. Nat. 54, 220–246.

———, 1920. Study of the constitution of the germ-plasm in relation to heredity. Carnegie Inst. Year Book, No. 19, 329–331.

MORGAN, T. H., A. H. STURTEVANT, and C. B. BRIDGES. 1923. The constitution of the germ-material in relation to heredity. Carnegie Inst. Year Book No. 22, 283–287.

STURTEVANT, A. H. 1920. Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *Drosophila melanogaster*. Genetics: 5, 488–500.

———, 1921. Genetic studies on *Drosophila simulans*. II. Sex-linked group of genes. Genetics: 6: 43–64.

———, 1921. Genetic studies on *Drosophila simulans*. III. Autosomal genes. General discussion. Genetics: 6, 179–207.

———, 1921. A case of rearrangement of genes in *Drosophila*. Proc. Nat. Acad. Sci. 7, 235–237.

FREQUENCY OF BAR REVERSIONS IN HOMOZYGOUS AND HETEROZYGOUS FEMALES

In order to determine whether the mutant, Bar eyes, mutates to normal (round eye) more frequently in different stocks, a few females of the stock of Bar were first out-crossed in pair matings to other stocks. A Bar female was mated in one case to a wild-type male of the New Hampshire stock, another to a Kushla wild stock, and four other Bar females were mated to males from other mutant stocks. The offspring consisted of heterozygous Bar females and Bar males, except in the rare cases of non-disjunction or of mutation when normal males and pure Bar females are expected. Out of 475 F_1 offspring, three males with normal eyes were found. Now, if any of these males arose through the fertilization of a no-X, non-disjunctional egg by an X-sperm, the XO males so produced would be sterile as previous experience with such males has invariably shown. Two of the three males were, in fact, found to be sterile and must have arisen through primary non-disjunction. The other male bred to a not-Bar (normal) eosin female gave normal-eyed daughters (and eosin sons) showing that he carried the gene for normal (or full or round) eyes, and since he was fertile it must be supposed that he arose as a mutation in one of the Bar chromosomes of his mother.

In order to be able to distinguish by inspection the exceptional (non-disjunctional) from the mutational (full-eyed) males in the offspring, red eyed Bar males were mated each to an eosin eyed female. If a full-eyed son arises from non-disjunction, his eye color will then be eosin; but, if he has arisen by reversion of Bar to round eye, his eye color will be red. In this case a mutated male can be identified as such at once by inspection, without the need of testing whether he is sterile or not.

Six Bar eyed F_1 males, from the six matings described above, were mated (in pairs) to eosin females. Out of 3,866 sons there were 15 Bar males with red eyes. These must have been non-disjunctional males. Out of 4,086 daughters there were 4 eosin full-eyed exceptional (non-disjunctional) daughters. There was not a single mutation to round eye amongst the 7,952 flies. There reappears in these results something that had been noticed several times before (Bridges 1916, Safir 1920), namely, the excess of males amongst primary non-disjunctional flies. This is interpreted to mean that when non-disjunction occurs both X's are lost (in one way or another) more often than they are retained in the egg.

In the absence of reversion of Bar to round in the experiment it is possible to draw only a negative conclusion from this evidence, namely, that fewer Bar reversions occurred than happened in other stocks, but the numbers are not large enough to prove that this is significant.

In order to find out whether, after reversion from Bar to round has taken place, the Bar descendents of such a fly that had been bred to Bar stock are more likely to revert again from Bar to round, the following experiment was carried out. A round eyed male that had arisen in Bar stock by reversion was crossed to Bar females from three pair cultures. The heterozygous Bar females were then mated to their Bar brothers. Some of the Bar offspring were next mated together and gave 167 Bar sons and daughters, with no reversions. In another case a heterozygous Bar female that had appeared in pure Bar stocks was utilized. She was bred to a Bar brother and several cultures were reared from the Bar offspring. In all there were 831 Bar sons and daughters and no reversions. This number is not sufficient to show whether the percentage of Bar reversion is, or is not, increased, but it suffices at least to show that no marked increase of reversion resulted.

An experiment was also planned to find out whether the reversion of Bar to normal occurs more frequently in the heterozygote than in the homozygous form. In order to do this it became necessary to block in the region on each side of Bar in such a way that if a mutation occurred in this region it could be detected. There are several ways in which this can be done.

$$\frac{+\qquad B\qquad +}{f\qquad +\qquad fu}\qquad\qquad \frac{f\qquad +\qquad fu}{}$$

Diagram 1

(1) When a Bar female is crossed to a forked fused male, the daughters will have Bar in one X-chromosome and forked fused in the other X. The forked gene lies 0.2 unit to the left of the Bar locus and the fused gene 2.5 to the right of Bar, (diagram 1).

Double crossing over in a distance of 2.7 units is precluded, hence, if this female, bred to forked fused male, produces a son that is normal and not-forked and not-Bar (at the same time) the Bar gene must have mutated to normal. Even if one crossover occurs between forked and fused (on one or the other side of Bar) there would be a record of this event for the resulting chromosomes would be respectively, +B fu and f++, or else ++fu and f B+, and the son that received one or the other of these chromosomes would obviously be a crossover. Any son that had round eyes and was neither forked nor fused must be the result of reversion of Bar to round eye.

The offspring of mating such a female to forked fused males are of four kinds (disregarding the rare crossovers just mentioned), namely + B + ♀, f + fu ♀, + B + ♂ and f + fu ♂. The experiment may be repeated again by mating the heterozygous Bar daughters to the forked fused brothers.

(2) Another way of performing this experiment and the one actually utilized is as follows. A Bar female is made up that has forked and Bar in one X, and fused and Bar in the other X-chromosome (diagram 2).

$$\frac{f\qquad B\qquad +}{+\qquad B\qquad fu}\qquad\qquad \frac{f\qquad +\qquad fu}{}$$

Diagram 2

Such a Bar female does not show either the forked or the fused character because the normal allelomorph of each is carried in the other chromosome. Her sons, each of which gets one or the other X-chromosome will be, for the most part, either forked Bar or fused Bar. Reversion to round in either chromosome will be indicated by the presence of forked or of fused. It is impossible to recover both chromosomes, since one or the other will be eliminated in the polar body of the egg in each case, but the kind of reverted males to appear should at least, it was supposed, indicate whether reversion occurred more frequently in one or in the other. The sequel, however, gave a somewhat different outcome as will be shown. The female in question was mated to forked Bar males and sometimes to fused Bar males. The kinds of sons that resulted are shown in table 1.

TABLE 1

	♀	f B ♂	B fu ♂	B ♂	f B fu ♂	N ♂	f fu ♂	het. B ♀	Gyn.	Mutants
TOV.......	241	118	106	1	0	0	1	1		
	240	90	55	1	8					
	268	115	78	1	1					
	69	24	22	0	0					
	306	131	135	3	1	1	0	0		
	389	133	134	5	3					
	154	74	76	1	1					
	359	99	81	12	2	1	0	0		
	100	49	35	2	0					
	66	36	11	0	1					
	542	249	204	9	4					
	190	94	70	2	1					
	203	80	58	1	0	1				
	205	140	43	8	0					
	196	88	59	5	2					
	77	43	2	1	0					
	98	39	14	1	0					
	169	76	76	9	1					
	77	41	17	4	0					
	267	116	102	5	3					
	91	30	20	0	0					
	182	66	29	2	0					
	230	64	51	5	0					forked ♂
	241	126	80	5	0	0	1	0		1
	266	95	114	2	0	1	0			
	154	74	76	1	1					
	57	29	13	0	1					
	209	68	48	5	0					
	70	28	16	1	0					
	154	31	46	7	0					
	122	35	45	0	1					
	70	24	18	1	0					
	135	48	41	0	0					
	197	61	40	7	0					
	157	52	57	0	0					
	158	62	45	4	0					
	70	27	20	2	0					
	162	53	37	4	0					
	61	17	3	0	0					
	214	81	34	2	0					
	117	44	26	0	0					
TOVeeo$_3$...	78	28	29	3	1					
	76	27	6	2	0					
	58	38	20	0	1					
TOVeeo$_4$...	36	17	7	0	0					
	106	47	39	1	1					
	163	59	54	3	2					
	109	42	29	1	0					
TOVeeo$_5$...	56	30	16	0						
	42	15	5	0						
	78	36	15	1						

TABLE 1—*Continued*

	♀	f B ♂	B fu ♂	B ♂	f B fu ♂	N ♂	f N fu ♂	het. B ♀	Gyn.	Mutants
	43	19	1	2						
	115	51	29	3						
	137	88	20	3						
	129	31	33	0						"Ultra"B♂
	115	56	33	2	1					1
TOVeeo$_6$...	101	45	32	1	3					
	15	6	5	0						
	49	14	12	0	2	1				
	68	25	16	1						
	10	4	1	0						
	33	14	15	1						
	33	10	2							
	67	34	8							
	61	20	17							
TOVeeo$_7$...	11	4	2					1		
	23	5	1					0		
	87	43	23	1	2			0		
	74	28	21	1	1	1		5		
TOVeeo$_8$...	63	30	13		1					
	54	35	6							
	154	58	28	3	2					
TOVeeo$_9$...	85	35	31	3	1					
	69	30	11							
	85	37	9	1		1				
TOVeeo$_{10}$...	6	5	0	0						
	21	6	3	1						
	32	8	0	0						
	125	54	27	2						
TOVeeo$_{11}$...	27	18	12	0	1					
	118	24	3							
	94	43	9	1						
	139	68	30	1	3					
TOVeeo$_{12}$...	105	42	12	1						
	68	22	3	1						
	63	30	8	1						
	105	44	3	1						
	73	31	10	5						
	96	34	13	1						

It may be stated at once that the experiment as planned did not give any evidence in support of the view that reversion of Bar to normal is more frequent than in heterozygous Bar stock (with the correction made for the double possibility, as it then seemed, in the pure Bar stock when both X's carry Bar). The experiment did lead finally to a discovery as to the nature of what happens when the reversion occurs (*see* Sturtevant, A. H., and Morgan, T. H., 1923. Reverse Mutation in the Bar Gene Correlated with Crossingover. Science, LVII, June 29, 1923, 746).

The two non-crossover classes of males in these results (table 1) are f B and B fu. There are also four expected classes of single crossover males, but phenotypically there are but two since in each case one of the crossovers between forked and Bar gave the same phenotype as one of the other crossovers (diagram 3).

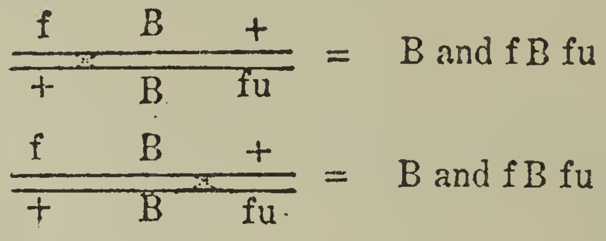

Diagram 3

Of course if reversion occurs in either X-chromosome, the resulting full eyed type may also be included in one of the crossovers. The fact that crossing over has occurred will, however, also be indicated by the change of linkage relations with forked or fused or both. The expected full eyed individuals would then be, theoretically, forked full eye, or fused full eye (non-crossover) or full eye or forked full eye fused (crossover). In fact, however, all the full eyed flies that appeared belonged to one or the other of the two latter classes, which means that whenever reversion of Bar to full eye took place, crossing over had taken place.

There are a few isolated records in these tables that must be regarded as exceptional in one way or another, but one instance may be mentioned that, had we not a clue from other sources, would have been extremely puzzling. In certain cultures, seventeen in number, in the later series from continuous breeding (as stated above) there were a large number of Bar males, as shown in table 2. These were no doubt due to "reversion" of forked to normal due to a modifier that not infrequently appears. At the time, these aberrant results were recorded but the flies were not tested. In the light of later evidence there can be little doubt as to their interpretation.

Table 2

♀	f B	B fu	B	f B fu	+N+♂	f N fu ♂	het. B♀
208	152	67	37	1	..	..	..
55	18	7	7	0	..	..	..
212	107	80	15	2	..	..	..
345	138	88	48	3	..	..	..
220	67	38	15	0	..	..	..
233	99	78	15	0	..	..	..
193	61	23	22	0	..	..	..
155	78	21	13	1	..	..	..
332	111	100	28	1	..	..	..
87	24	33	8	0	..	..	..
154	45	15	21	0	..	..	..
152	47	43	10	1	..	..	..
291	88	52	12	1	..	..	..
44	16	0	15	0	..	..	..
131	60	19	31	0	..	..	..
162	70	26	11	2	..	..	..
203	68	27	26	0	..	..	..

INFLUENCE OF ATMOSPHERIC PRESSURE

At a time when the peculiar mutation of the "Bar gene" to normal was not yet analyzed, several attempts were made to see whether the reversion could be affected by environmental changes. Now that the nature of the change has been made out the data may have still some slight interest as bearing on the possible effect of particular environmental changes on the crossing over that changes Bar to normal.

Cultures of forked Bar and forked Bar fused larvæ and pupæ were put into a bomb into which air could be pumped and the pressure increased. Two cultures (May 11, 1921, at 11:30 a. m.) were subjected to a pressure of 100 pounds. At 4:45 p. m. the pressure had sunk to 20 pounds. It was then raised to 100 pounds (at 5 p. m.). Next morning the pressure was down to 9 pounds at 9:50 a. m. when the cultures were removed. Several flies were found present in four of the five bottles, that had hatched under pressure. These as well as those hatching in the next few days were reared in pair cultures.

Nineteen pairs from A gave 1,096 Bar males and 1,022 Bar females. In addition there was one reverted Bar male with full eyes.

Seven pairs from B gave 693 Bar males and 660 Bar females. In addition one full-eyed male and a heterozygous Bar female.

Five pairs from D gave 112 Bar males and 82 Bar females.

Sixteen pairs from E gave 1,329 Bar males and 1,334 Bar females. In addition there were one full-eyed male and one full-eyed female.[1] In one experiment flies from A were mated to flies from B. There were 657 Bar males and 623 Bar females with no reversions.

All the cultures taken singly ran fairly evenly for sex ratios, and the totals given above are made up of rather homogeneous data. The experiment was not made in such a way that non-disjunction could be recorded. In all there were 7,608 offspring and 5 reversions or 1 to 1,521 flies. In a second experiment less pressure was used but as it became lower it was renewed more often than before; thus

May 12, 11:30 a.m. to 50 pounds at 2 p. m. 21 pounds.
2:00 p.m. to 50 pounds.
5:15 p.m. to 50 pounds 5:40 p.m. 40 pounds.
5:45 p.m. to 54 pounds 10:00 p.m. 29 pounds.
10:00 p.m. to 50 pounds May 13, 9 a.m. 5 pounds.

The cultures were removed May 13, 9 a. m. In one culture 12 flies had hatched (Z′). These and those that emerged from other cultures were mated in pairs that gave the following results:

From one culture (V′) that began to hatch on May 21, four pairs were made up and three more with mates from one of the preceding lots. These produced 555 Bar sons and 565 Bar daughters, and in addition one heterozygous Bar female.

From another culture (X) seven pairs gave 723 Bar males and 693 Bar females. In addition one full-eyed male was present, and 3 females and a male that were Bar and had a notch in the ends of the wings.

From another culture (Y) seven pairs gave 142 Bar males and 132 Bar females.

From culture Z seven pairs gave 440 males and 436 females; in addition one full-eyed male. In two of the cultures seven notch winged[2] Bar-eyed flies appeared in each. From culture Z^2 eleven pairs gave 623 Bar males and 637 Bar females. In addition one full-eyed male (and seven notched).

---

[1] This female must have been either extreme Bar or else due to contamination.

[2] Probably not the mutant notch.

In all there were 4,946 Bar flies and 4 cases of reversion or 1 in 1,236 flies.

Taking both experiments together there were 12,654 Bar flies and 9 reversions or 1 in 1,406.

These results comprising over twelve thousand flies do not give a ratio of reversion appreciably different from that found in other stocks under normal environmental conditions. More than this can not be stated, for with so small an expectation of reversion much larger numbers would be necessary to show whether the results are significant.

HIGH TEMPERATURE ON LARVÆ AND PUPÆ OF *DROSOPHILA*

The sensitiveness of *Drosophila* to temperatures above 30°C led to the following attempts to examine the effects of higher temperatures for short periods. Water warmed slightly above the desired point was poured out into a new, warmed dish and mixed with cooler water until exactly the desired temperature was reached. By subsequently adding warmer or cooler water and stirring the mixture the temperature for the short interval

Table 1

		♀	♂	N ♂	
Larvæ...	36° for 5 min.	60	65	0	..
	40° (?) 5	70	71	1	..
	36° 5	44	47	..	1 Broad B ♂
	38° 5	46	44	..	
	38° 5	41	29	..	..
	38°–39° 5	53	76	..	..
	36°–37° 5	30	28	..	..
	37°–39° dry 5	116	136	..	..
	36°–38° 5	26	17	..	..
	40° 5	61	48	..	..
Pupæ....	40° 5	69	61	..	..
	40° 5	51	56	..	..
	41° 5	42	48	..	..
	39° 5	84	87	..	..
	38°–39° dry 5	46	60	..	..
	40° 5	54	59	..	..
	39° 5	17	29	..	..
	38° 5	125	130	..	..
	40° 5	73	96	..	..
	38° 5	131	149	..	..
	40° 5	22	12	..	..
	38°–39° 5	11	7	..	..
	40° 5	56	50	..	..
	41° 5	31	26	..	..
	39° 5	246	196	..	..
	40° 5	49	73	..	..
	36° 5	82	61	..	..
	36° 5	76	80	..	..
	37°–39° dry 5	124	104	..	..
	39°–41° 5	41	48	..	..
	38°–39°	21	25	..	..
		1,998	2,018	1	1

required could easily be held within a degree. Larvæ and pupæ of Bar stock of any desired age were dropped into the bath and afterwards removed to food-bottles. The number of offspring that was produced and their sex was recorded (table 1).

Out of 4,017 offspring there was only one reversion to normal. There was no evidence that any effect was produced on the sex cells of the treated larvæ. No other mutants were recorded. The sex ratio was not changed; in fact unless both X's of the female had been injured there was no expectation of any alteration. At most such an effect would be expected on a few cells and if this was in later stages a change would not be observed; had it taken place in an earlier stage the affected cells might not proceed normally. If the X-chromosomes were changed to give recessive mutants these would be looked for in the males, but if in other chromosomes, the effect in the first generation would be covered up by the normal allelomorph in the other chromosome. Pair matings would in time reveal such recessive genes, but this was not tested.

Lethal mutations in the X-chromosome would not be revealed in the first generation for, were such an X-chromosomal effect produced, once in a while, such eggs, if fertilized by a sperm with normal X, would give a normal female, and if the egg were fertilized by a Y-sperm the zygote would die. The loss of a few males would not appreciably affect the sex ratios.

DELAYED MATING

Newly hatched virgin females were isolated for a week or more and then were mated. Such females are supposed to hold back their eggs, for a time at least, until fertilized. Under such circumstances not only the delayed, mature egg, but the younger eggs in the ovary might also be affected. Several tests were made (1) to determine whether non-disjunction of the X-chromosomes would occur; (2) to determine whether linkage was influenced; (3) to find out if the sex ratio was affected. The experiments were defective in two ways. It was not determined in all cases that the female had not laid some of her eggs. The distension of the abdomen showed, however, that eggs were being retained. It was not determined whether the first laid eggs were fertilized and developed, or whether they were laid without being fertilized after mating had taken place. Since, however, the results did not give any evidence of anything unusual taking place the fate of the first laid egg is not here a matter of importance.

The first experiment gave evidence as to the sex ratio and the occurrence of non-disjunction if it had taken place. Wild type virgin females were isolated for 14 days. Food was, of course, supplied to them. Each was then mated to a few forked Bar males and given new food. They gave the following results:

Hetero. Bar ♀	57	18	63	25	87	15	20
N ♂	59	11	35	15	57	8	12

There were no non-disjunction flies. The cause of the deficiency of males in several bottles is not evident. The bottles were run to a finish in order to get any late hatching males.

In another series the virgin females, forked Bar, were isolated for 13 days

(July 10–23). They were then each mated to vermilion males and gave the following results:

Hetero. Bar ♀	f B ♂	forked ♂	v♂	N♀
64	47	..	..	1
63	85	..	..	..
55	56	..	..	..
49	46	..	..	..
93	111	..	..	..
90	83	3	1	..
78	48	..	..	..

In only one culture were the males suspiciously deficient. In the first culture there was a normal eyed female. Her presence can possibly be explained by reversion in an X-chromosome of the forked Bar female.

In the sixth culture there were three forked males. The occurrence can only be explained as the result of Bar reversion. This is unexpectedly high for the number of flies involved.

In the same culture also there was one vermilion male that can be explained as arising from a non-disjunctional egg by an X-bearing vermilion sperm.

Another similar experiment was made in which the forked Bar females were isolated for 12 days:

Hetero. Bar ♀	f B ♂	B ♀
18	11	....
48	36	....
33	31	....
38	27	....
57	54	....
52	47	....
61	58	1 Double Bar over normal.

In the seventh culture a Bar female appeared. She can be accounted for possibly on the assumption that she arose from a double Bar gamete (due to crossing over between the forked Bar chromosome of the mother) fertilized by an X-sperm.

Several other experiments were made with females whose mating was delayed for different times as follows:

♀		B ♀	B ♂	Hetero.B ♀	N ♂
10/15	11/9	42	24	....	....
10/15	11/9	53	64	....	....
10/15	11/2	119	71	....	....
10/23	11/9	49	48	....	....
10/16	11/2	88	104	....	....
10/15	11/9	8	9	....	....
10/15	11/2	58	46	0	1
10/23	11/13	71	105	....	1
10/16	11/2	125	108	....	....
10/15	....	61	58	....	....
10/19	11/23	96	79	1	0
		—	—	–	–
		870	716	1	2

The possible effect of delayed fertilization on crossing over was tested in a three point experiment involving the genes Dichæte (D) and scarlet (st) and spineless (ss). Virgin females (Dichæte peach spineless) were mated to scarlet peach males. Both parents were pure for peach. The peach F_1 females that were Dichæte spineless in one third chromosome and scarlet in the other were isolated for 25 days, and then mated in pairs to scarlet spineless males. It is to be noted that while these F_1 females were 25 days old they had probably laid eggs during their isolation and that the actual eggs that were fertilized need not be supposed to have been ripe eggs that were delayed for 25 days. Nevertheless it was possible that some delay occurred in egg laying and this might possibly have an influence on the crossing-over in them. It was this point that the experiment was devised to test. The relation of the genes involved is shown in the next diagram that represents the history of the third chromosome pair in the F_1 female that was tested. In the table the data are arranged into non-crossovers, into crossovers at the first level (between D and st), at the second level (between st and ss) and double crossovers.

$$\frac{D' \quad + \quad pe \quad ss}{+ \quad st \quad pe \quad +}$$

First Crossover level   Second Crossover level

Non-Crossovers		Crossovers at 1st level		Crossovers at 2nd level		Double Crossovers	
D ss	st	D st	ss	D	st ss	D st ss	Normals
103	106	2	1	12	11	2	....
98	91	4	4	14	14	2	....
77	112	1	1	10	11	....	....
57	84	1	4	6	10	3	1
86	119	1	2	5	16	1	1
421	512	9	12	47	62	8	2

The values give 2.9 crossing over at the first level and 11.9 at the second level.

At the same time that the above matings were made the same cross was repeated and the F_1 females were mated at once with males. Three pairs were kept under the same environmental conditions as the former, and, in a sense, served as a control giving the crossover values for the particular stocks that were used in the delayed experiment. The next table gives the results.

Crossing over in the first region gave a value of 4.3, in the second region 16.7. Comparing these with the values for the delayed females we find:

	First level	Second level
Delayed..........	2.9	11.9
Control..........	4.3	16.7

D ss	st	D st	ss	D	st ss	Doubles	
						D st ss	Normals
37	39	1	2	4	8	4	0
79	93	1	3	34	14	1	0
58	65	3	1	12	19	1	1
97	88	5	5	13	18	7	2
124	113	5	2	16	22	2	0
74	72	1	2	11	19	1	2
125	118	6	4	21	10	1	0
594	578	22	19	111	110	17	5

There is less crossing over in the delayed females, a difference that is doubtfully significant. It is true that this control is higher than the recombination values for these regions given in the general results of other experiments (*viz.*, D st 3.4 and st ss 13.6) but this is expected since the latter include also later hatched flies.

While the values for the delayed females are still somewhat lower than the general recombination values, the differences may not be significant. Further comparisons would not be profitable until it could be found out how many unfertilized eggs have been laid by each of the females tested. Only in this way can it be determined with what part of the normal (or regular) output the delayed eggs are to be compared. Furthermore, even if all the ripe eggs are not deposited by a female, it is quite possible that some of her retained eggs may not be capable of development. Again there is the possibility of the effect being produced by the somatic aging of the tissues of the female or other physiological changes connected with age, rather than being due to the influence of the retention of mature eggs or the processes taking place (crossingover) in the immature eggs. Until this complex situation is further analyzed the results mean no more than that a slight change was recorded as having taken place.[1]

---

[1] When the experiment was made a third control was made in which a second brood was obtained from one or from the other of the foregoing lots of females. That is, the pairs were carried over into new bottles and their offspring classified. The notes made at the time are not now quite clear as to whether these second broods came from the delayed females (which I think is the more probable interpretation) or from the control. The results obtained gave still lower values than for the delayed eggs. The totals were as follows:

D ss	st	D st	ss	D	st ss	Doubles	
						D st ss	normals
368	313	1	6	41	53	2	2

The value at the first level is 1.3; that at the second level 11.7.

VIII

COMPOSITES OF DROSOPHILA MELANOGASTER

By L. V. Morgan

With four text-figures

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COMPOSITES OF DROSOPHILA MELANOGASTER

By L. V. Morgan

INTRODUCTION

Morgan and Bridges (1919) found that nearly all of the gynandromorphs of *Drosophila melanogaster* can be explained on the hypothesis of "simple single elimination." An ordinary egg, containing one X-chromosome is fertilized by an X-bearing sperm and the XX-individual starts as a female; at some (usually an early) division of the developing egg, a daughter half of one of the X-chromosomes "fails to reach its pole and is lost in the mid-plate or in the cell-wall," leaving a part of the embryo with only one X-chromosome and hence male. The result is a gynandromorph which may be schematically represented by one of two diagrams (fig. 1, a and b), since the eliminated chromosome is sometimes the maternal chromosome (Xm) and sometimes the paternal (Xp). The female region of a gynandromorph so produced is expected to be like a regular female, as has been proven by the phenotype and in many instances by the offspring of gynandromorphs that have inherited X-chromosomes carrying mutant genes and have bred as females. The characters shown by the male region of a heterozygous gynandromorph will depend upon which chromosome remains in the region after elimination. The male region is expected to be either like one of the regular kinds of males or like the father, depending upon which chromosome remains in the region after elimination of the other chromosome. The chromosomal constitution of the region is expected to differ from that of the regular male by the absence of a Y-chromosome. Negative evidence of the XO constitution of the male region has been found in the sterility of gynandromorphs with apparently normal male genital apparatus, sterility being a characteristic of XO males. Positive genetic evidence has recently been obtained from three gynandromorphs, found by Stern (1926), which were homozygous for the sex-linked character bobbed. He has proved that a normal allelomorph of bobbed is carried by the Y-chromosome; this explains why the males of bobbed stock do not show the character although they transmit it through the X. The male region of the gynandromorphs, how-

Xm1 X'p	Xm1 [Xm1] X'p X'p	Xm1 Xm1 [X'p] X'p
z.	a.	b.
X'm2 X'p	X'm2 [X'm2] X'p X'p	X'm2 X'm2 [X'p] X'p
z'.	a'.	b'.

Fig. 1—Diagram of sex-chromosomes of the four expected classes of gynandromorph from a regular single nucleus by single elimination. z, z', zygotes, a and a', gynandromorphs by elimination of a maternal X. b and b' by elimination of the paternal X. (In a backcross X'm and X'p are alike.)

ever, was bobbed as are also non-disjunctional XO males of bobbed stock, showing the absence of a Y-chromosome from the male region.

Nine composite flies described by Morgan and Bridges could not be explained by a process of simple single elimination; among these were both unisexual mosaics (males and females) and gynandromorphs. These all differed from the many simple cases in that they showed the presence of two different X-chromosomes derived from the mother. If the two maternal chromosomes remained in the egg by non-disjunction, the zygotes after fertilization contained three sex-chromosomes and at some stage of development one, or, in most of the cases, two or even three daughter chromosomes must have been eliminated (*cf.* fig. 3, p. 270, and fig. 4, p. 284).

As an alternative explanation to that which requires non-disjunction followed by multiple elimination, the authors suggest that "Doncaster's observations on binucleated eggs of Abraxas, where both nuclei underwent separate reduction and fertilization, offer a simple explanation" (*see* fig. 2).

Xm1 or 2	IIm1 or 2	IIIm1 or 2	Xm1 or 2	IIm1 or 2	IIIm1 or 2
Xp or Yp	IIp1 or 2	IIIp1 or 2	Xp or Yp	IIp1 or 2	IIIm1 or 2

FIG. 2—Diagram of possible combinations of chromosomes in composites developed from an egg with two separately matured nuclei, fertilized by two spermatozoa (disregarding the IV-chromosome).

But they add: "On the other hand, it should be pointed out that there should have been at least as many autosomal mosaics as sex-linked mosaics produced by fertilization of binucleated eggs of heterozygous mothers; and this does not seem to be the case."

There were no data for the direct comparison of the frequency of autosomal mosaics and of gynandromorphs within the same experiment. It was therefore proposed that a study should be made of composites of all kinds from crosses in which the two large autosomes (II and III) and the X-chromosomes had been favorably marked for the purpose.

The result of the experiment was negative in so far that there was no positive proof that any one of the exceptional flies had been derived from two nuclei, and the indirect evidence in the questionable cases was in favor of elimination. It is now known, however, from other sources, that binucleated eggs probably do occur in *Drosophila.* Nevertheless, the combined data from the present experiment and from recorded work on *Drosophila* go to show that elimination has a wider application than the one first made use of to explain gynandromorphs, and that even multiple elimination may be more frequent than the very rare occurrence of binucleated eggs.

In presenting the evidence for this conclusion, "elimination" will be used in relation to the X-chromosomes only unless otherwise stated. "Composite" is used to denote an individual made up of, at least, two regions which have developed from cells not containing identical chromosomes, regardless of the process by which the difference in the chromosomal constitution of the two regions has come about; it includes, among many other types, gynandromorphs derived by elimination of an X-chromosome, and flies derived from two separately fertilized nuclei in the same egg, whether the flies are unisexual or gynandromorph. "Mosaic" is used when visible character differences are present in two regions. In the diagrams and tables, a system of letters has been used to distinguish the source of the chromosomes. Thus, Xm1, Xm2, Ym, IIm1, etc., stand for the maternal chromosomes, and Xp, Yp, IIp1, etc., for chromosomes received from the father. When the male and female regions are so distributed that a character does not affect any organ in a region in which it is known to be genetically present, that character cannot be diagnostic and its symbol is enclosed in square brackets.

1. EXPECTED FREQUENCIES OF DIFFERENT TYPES OF COMPOSITES

By chance elimination of a single X-chromosome from a regularly matured egg fertilized by X-sperm, the composites that would result would be gynandromorphs of which 50% would have in the male region the maternal X and 50% the paternal X. If the X-chromosomes of a homozygous mother carry different mutant genes from those carried by the paternal X, the gynandromorph offspring (if bilateral or otherwise favorably divided) will be mosaic for sex-linked characters, and it can be determined from the phenotype which chromosome has been eliminated (table 1, lower line). One of these phenotypes, namely the gynandromorph containing the paternal X in the male region, type a, could not be derived from a binucleated egg, but the other phenotype, type b, lends itself to both explanations.

Table 1— $\frac{X}{X}\frac{\cdot II}{II'}$, *by* $\frac{X'}{Y}\frac{II'}{II'}$

		IIm or II′m in both nuclei (not autosomal mosaic)			IIm in one nucleus, II′m in the other (autosomal mosaic)		
from a binucleated egg		X-sperm and Y-sperm sex-linked mosaic gynandromorph 2	two Y-sperm male (not detectable) 1	two X-sperm female (not detectable) 1	X-sperm and Y-sperm sex-linked mosaic gynandromorph 2	two Y-sperm male 1	two X-sperm female 1
from one nucleus by elimination of an X	sex-linked mosaic gynandromorph a. X′p in male region 4	sex-linked mosaic gynandromorph b. Xm in male region 4					

Ratios of the kinds of composites to be expected from the simplest marking of the X-chromosomes and of one pair of autosomes that will give any diagnostic classes.

Flies derived from two separately fertilized nuclei may be autosomal mosaics if an autosomal mutant gene is present, and may be either unisexual or gynandromorph. If, to take a simple example, a fly is derived from a binucleated egg in a backcross for one recessive character in autosome II or III, the chances that it will be an autosomal mosaic are even. The two possible zygotes, in respect to sex-chromosomes, may be called XmXp and

XmYp. Given the first in one nucleus, the chances are even that the other nucleus will be like it (producing a female) or unlike it (producing a gynandromorph). Given the second zygote in one nucleus, the chances are even that the other nucleus will be like it (resulting in a male) or unlike it (a gynandromorph). The expected ratio then for composites developing from two nuclei in a backcross for one autosomal mutant character (in II or III) is: 4 gynandromorphs to 4 unisexual composites (2 males and 2 females), of which half of each kind are autosomal mosaics. (Compare table 1, upper row.) The male and female which are not mosaic will be indistinguishable from the regular males and females of the backcross, leaving 2 diagnostic unisexual mosaics to 4 gynandromorphs, 2 of which are also autosomal mosaics.

If in the autosomal backcross the X-chromosomes are marked in the way that has been assumed for the hypothetical case of gynandromorphs by elimination, then the two gynandromorphs in the last ratio that are not autosomal mosaics will be of the same phenotype as half of the gynandromorphs that would result by elimination, namely, the 50% having Xm in the male region. This ambiguous phenotype common to both origins is not directly useful in estimating the relative frequency of the two modes of origin, but the numbers of the composites that are in accord with only one or the other explanation can be compared.

In the work on *Drosophila,* often only one pair of chromosomes has been marked in a given experiment so that the data for comparison of frequency of types of composites must be collected from different experiments and only a rough estimate of expectation can be made. For instance, if both origins were equally common and if the flies examined were equally numerous in two very usual kinds of matings, namely, those in which the parents are unlike for X and in backcrosses for an autosomal character, then equal numbers would be expected of two not ambiguous types, namely, gynandromorphs with the paternal X in the male region by elimination, and autosomal mosaics from binucleated eggs.

An analysis of the combined data from miscellaneous experiments, relating to simple gynandromorphs and autosomal mosaics is given in section 3. In section 2, new simple gynandromorphs without marked autosomes are described. The next section has been inserted for reference and can be passed over in reading the paper for an analysis of the data.

2. DESCRIPTION OF NEW GYNANDROMORPHS WITHOUT MARKED AUTOSOMES

Twenty-five gynandromorphs have been seen in a stock of eosin, homozygous bar (w^eB) XXY-females, mated to vermilion (v) males, which gave besides the regular red heterozygous bar-eyed females and eosin bar males, a high percentage of exceptional w^eB females and v males. The composites have been observed and recorded by Miss E. M. Wallace, but the records have not yet been published.

All but one of the gynandromorphs were offspring of exceptional XXY-females mated to exceptional males. Since the mutant characters of the parents affected only the eyes, ten heterozygous bar-eyed gynandromorphs, in which the entire head belonged to the female region, give no clue as to the chromosomal constitution of the male region and will therefore not be considered in detail. In regional distribution they differed; one was described as a male with female head; in four the abdomen was male and more or less normal, and in three at least of these all or part of one side of the thorax was also male. The other five were decidedly more female than male; *e. g.*, the head female and the rest of the gynandromorph bilateral, or only one side of the thorax or part of the abdomen male. One or two bred as females and two failed as males.

No. 3 (1), the offspring of a pair of exceptional flies, had, on one side, a round red eye, a sex comb and a shorter wing; on the other side, a red heterozygous bar eye. If an egg with an X-chromosome (Xm) bearing genes for the characters eosin and bar had been fertilized by a sperm containing a vermilion-bearing chromosome (Xp) and at some division the maternal chromosome (Xm) had been eliminated, the result would be accounted for, allowing for a peculiarity of the character vermilion. Sturtevant (1920) discovered that when the gene for vermilion is genetically present in the male region of a gynandromorph, the eye is (sometimes at least) not vermilion but red, apparently wild type. (In diagrams of flies so explained, the symbol for vermilion is marked by an asterisk.) The binuclear explanation can be applied to No. 3(1) if one nucleus had received a maternal X-chromosome (with or without the maternal Y-chromosome), and the other (like one of the exceptional classes of eggs) the maternal Y-chromosome, and if both had been fertilized by X-sperm as represented in the diagram on the right.[1] The exceptional eggs are however comparatively so infrequent that the second explanation is improbable.

w^e B		w^e B	
———————— Xm		———————— Xm	.... Ym
(.............. Ym)?	(.............. Ym)?	(.............. Ym)?	
v	v*	v	v*
———————— Xp	———————— Xp	———————— Xp	———————— Xp

The parentage and the explanation of the next two flies are like those of No. 3(1). No. 12b was roughly an antero-posterior gynandromorph. The head was wild type except the lower half of the right eye which was narrow (heterozygous bar?) but also red; a drawing made from the fly shows that the right ocellus was missing which suggests that an abnormality of the right side of the head may have accounted for the shape of the right eye, in which case the eye was not-bar, and the head was altogether male, genetically vermilion. The fly had two sex combs, and wings of equal length. The abdomen was female externally (except half of the next to last segment), and internally there was a complete normal female genital apparatus.

No. 15b was a nearly bilateral gynandromorph. The right side was male with a round red eye, a sex comb, shorter wing, shorter bristles, and with male coloring on the next to the last abdominal segment. The left side was female with heterozygous bar eye, no sex comb, longer wing and bristles. The eye of the male region as in other cases of the same parentage was not vermilion as would be expected if the exceptional behavior of vermilion were not known. In this case it is proved by the offspring that a vermilion-bearing chromosome was present in the female region together with one bearing genes for eosin and bar. The offspring of the fly when mated to a forked male were:

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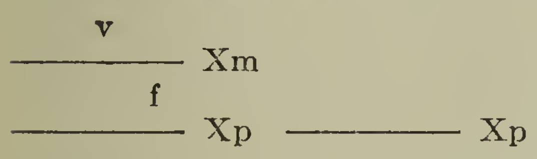
[1] Diagrams on the left side of the page represent flies as derived by elimination, those on the right as derived from two nuclei.

Females		Males					
het. B	wild type	we B	v	we v	B	we	v B
32	35	20	24	5	3	5	5

Since the eye of the male region was not eosin or bar, it is evident that the vermilion bearing chromosome was the one present in the male region (barring the unlikely possibility of a mutation).

No. 2 is described as female on one side and male on the other side, with one sex comb; the eyes were round and red. If half of the head was included in the female region, the maternal X-chromosome may have been one from which the gene for bar had been lost by crossing over (Sturtevant 1925). In the high non-disjunctional stock, there were the usual not infrequent "reversions" to normal which are found among homozygous bar flies. But the head is sometimes entirely of one sex in an otherwise bilateral gynandromorph; if this was true of No. 2, both eyes may have been female or they may have been male and of wild type red color in the genetically vermilion male region. The last assumption is the most probable, but all three admit of the same explanations of the gynandromorph as were given for the last three flies, namely elimination or binucleuted egg.

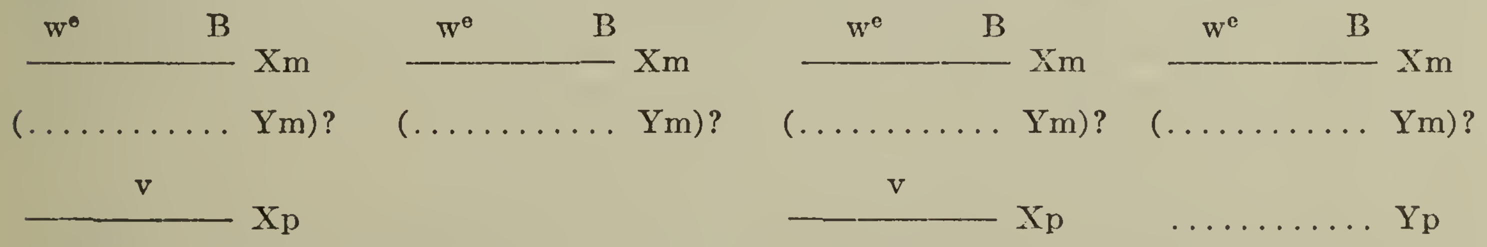
The mother of the next gynandromorph was a wild type female, the daughter of an eosin bar XXY female and a vermilion male. The mother had one chromosome with a deficiency for bar (Morgan 1926) and one vermilion-bearing X. She was mated to a forked male. The gynandromorph offspring, No. 13a12a, was a red eyed fly which had received the vermilion-bearing chromosome from the mother and a forked-bearing chromosome from the father. The right side was forked throughout except the genitalia; a sex comb was present, the wing was short, and the abdomen male. The left side was female not-forked, with no sex comb, a long wing and female abdomen. The fly had one normal ovary and perhaps also a small rudimentary testis. It was mated to a forked bar male and produced:



Females		Males			
het. B	f het. B	v	f	vf	wild type
80	57	78	49	15	18

Elimination of Xm is, in this case, the only explanation, if the mother was a regular XX female; if, however, the mother of the gynandromorph had received a Y-chromosome from her mother the binuclear explanation can be applied, assuming that one nucleus was of the exceptional class that contains a Y-chromosome.

For the next seven flies (all offspring of exceptional males and females) elimination of the paternal X or origin from a binucleated egg equally well satisfy the conditions, as represented in the next diagram.



No. 6a was female with heterozygous bar eyes except the ventral part of the right eye, which was described as eosin bar (in a drawing an "eosin?" spot is figured, but it lies above the ventral narrowest part of the eye). The male region included otherwise only the fourth and fifth dorsal tergites and spiracles on the right, and four ventral plates. Internally the abdomen was normal female. The fly bred as a female, having genes for w^eB in one chromosome and for v in the other.

No. 3(2) had on one side a red heterozygous bar eye and on the other side the eye was "narrow red and male eosin bar." There is no further description.

No. 32/8c similarly had a left red heterozygous bar eye and a right eosin bar eye with a red spot; the right ocellus and half of the median ocellus were eosin. The fly was mostly female with wings of equal length, but there was a small sex comb on the right leg. It was mated, but failed to breed.

No. 32/4a was almost bilaterally divided. The male region on the left included a light eosin bar eye, except for a red patch posteriorly, and two eosin ocelli, the left side of the thorax with shorter bristles and a sex comb and shorter wing, and the left side of the abdomen. The right side was female with heterozygous bar eye. The genitalia were entirely female and also the internal genital apparatus.

In three of this group of gynandromorphs (Nos. 23a, b and 13b), the anterior parts were male, and both eyes were eosin bar. With no clue to the constitution of the female region a third interpretation is possible.

$[w^e]$ $[B]$ ———— Xm	w^e B ———— Xm
$[w^e]$ $[B]$ ———— Xm	
(………… Yp)?	………… Yp

If non-disjunctional XX-eggs were fertilized by Y-sperm elimination of an X from one region would produce the phenotypes. This is the only kind of interpretation by elimination applicable to two flies of the same stock with eosin homozygous bar eyes, both in the female region. The flies will be described later. In the description of No. 23a it is specifically noted that the color was probably the light eosin characteristic of male eyes. The fly had two sex combs; the abdominal plates were normal female on the right and male on the left, and on the left were male markings on the last two abdominal segments. The genitalia were female and there were two ovaries, and two dorsal and one ventral receptacle and no internal male parts.

The second, No. 23b, with two eosin homozygous bar eyes, also had two sex combs. The large bristles on the head and thorax and the wing are described as yellowish, but not the bristles on the abdomen. The next to last segment was male; otherwise the abdomen was female and also the genitalia. It was mated to a vermilion male and failed to breed.

The third, No. 13b, was largely male with eosin bar eyes, two sex combs and wings of equal length. The mother was heterozygous for bar deficiency but the gynandromorph developed from an egg that had received the maternal eosin bar chromosome, as shown by the presence of bar eyes in the region that was male. The abdomen was normal female in structure but with male pigment on the left side. The internal reproductive organs were normal female with fully formed eggs, and in the ventral receptacle were active sperm, but the fly failed to produce offspring.

No. 31c was an antero-posterior gynandromorph, with eyes that were male eosin but round. The parentage differed from that of the other gynandromorphs in that the mother had one X-chromosome that had "reverted" to not-bar and she was therefore heterozygous for bar. The gynandromorph had two sex combs and pos-

w^e ———— Xm	w^e ———— Xm	w^e [B?] ———— Xm	w^e ———— Xm
(………. Ym)?	(……….Ym)?	(………. Ym)?	(………. Ym)?
v ———— Xp		v ———— Xp	………. Yp

teriorly was female. Elimination of Xp would give the result. It was mated to several males and failed to breed. The binuclear explanation applies if it is assumed that the nucleus fertilized by Y-sperm had received a not-bar chromosome.

A few other gynandromorphs not previously described have been found from various sources.

No. A (*cf.* table 2) was a nearly bilateral gynandromorph whose mother was heterozygous for apricot (w^a). The father was eosin cut vermilion forked. The left male side was shorter than the right. On the left, the eye was apricot, the arista, the bristles and the wing were shorter than the corresponding parts on the right side where the eye was apricot-eosin compound. A small sex comb was present on the left side and none on the right, and the pigment of the abdomen and the genitalia on the left were male; on the right female, somewhat abnormal. The gynandromorph can be explained by elimination of the paternal X, or as having developed from two nuclei.

w^a ———— Xm1	w^a ———— Xm1	w^a ———— Xm1	w^a ———— Xm1
	w^e ct v f ———— Xp	.... Yp	w^e ct v f ———— Xp

A gynandromorph (No. 115a) was produced by a female which had one yellow-bearing chromosome, and one bearing forked and bar backcrossed to a forked bar male. The gynandromorph was female except the thorax on the left side. Both eyes were heterozygous bar; the female region was not-forked, there was no right sex comb and the right wing was longer. On the left side there was a sex comb, and shorter wing, and the thorax was forked dorsally and ventrally. The fly bred as a female like the mother, showing that it had received a yellow-bearing chromosome from her. Elimination of Xm or a binuclear origin explains the phenotype.

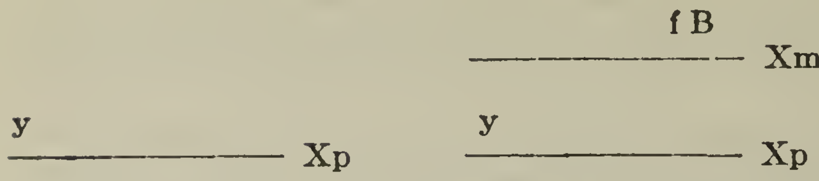
	y ———— Xm1	f [B] ———— Xm2	y ———— Xm1
f [B] ———— Xp	f B ———— Xp	.... Yp	f B ———— Xp

In No. 3.83c (of another stock) male and female regions were similarly distributed. The mother was yellow with two attached X-chromosomes but the gynandromorph received a single yellow-bearing chromosome from her and a Z-ple (sc ec cv v g f) chromosome from the father. The left side of the thorax was yellow and male with a sex comb and shorter wing and bristles. The eyes were wild type in color and so were the entire head, the right side of the thorax (with no sex comb and longer wing) and the abdomen. The offspring showed the fly to have been heterozygous for yellow and for Z-ple. The gynandromorph might have been derived by elimination of a paternal X, or from two nuclei.

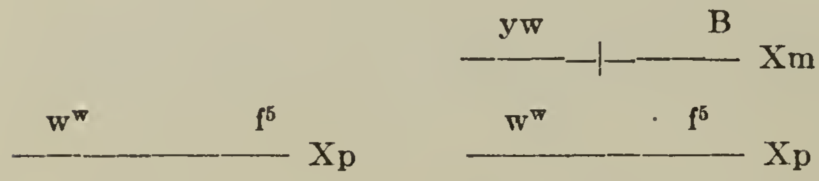
y ———— Xm	y ———— Xm	y ———— Xm	y ———— Xm
	Z-ple ———— Xp	.... Yp	Z-ple ———— Xp

No. 11.163 was the offspring of a forked female heterozygous for bar by a yellow male. The gynandromorph was female except the left side of the abdomen which was male and yellow from the mid-dorsal to the mid-ventral line; the genitalia were male and yellow and turned to the side. The female region was wild type in color; the eyes were heterozygous bar, there were no sex combs, the wings were gray, long

and equal; the last left leg was deformed. The only explanation is elimination of the maternal X.

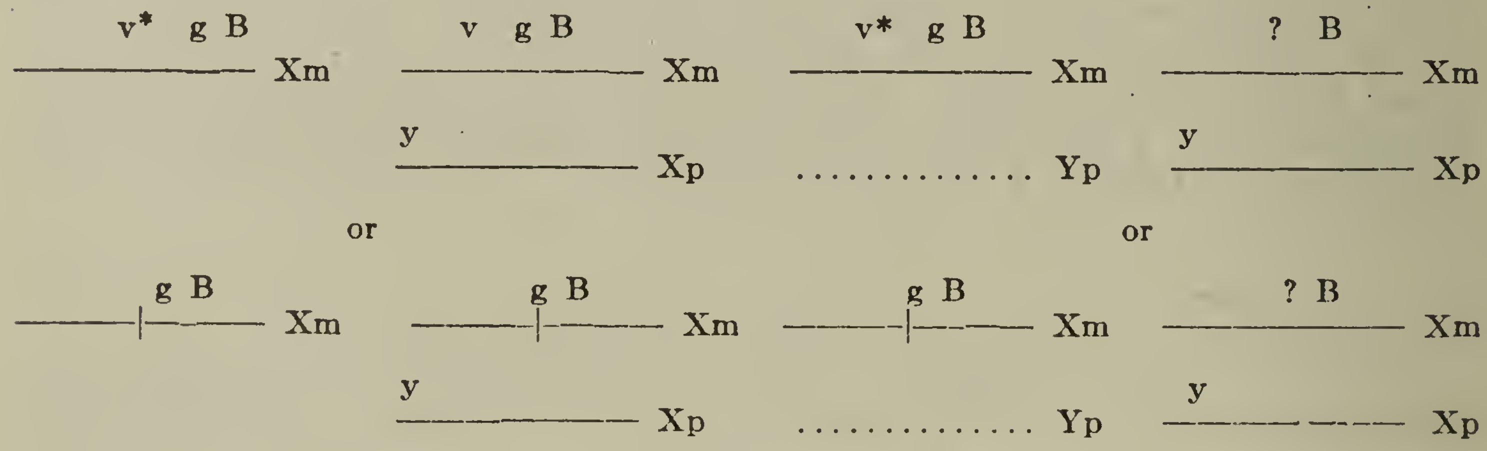


The mother of No. 12.176 was homozygous for bar and carried in one of her X-chromosomes also genes for yellow, white and forked; the father was wine and forked[5]. The gynandromorph was almost entirely female. Only the dorsal part of the head, the left antenna and eye and the anterior left leg, bearing a small sex comb, were male; these parts were forked, the eye was not-bar. The right eye was white-wine compound, heterozygous bar; the female region—thorax, abdomen and ventral side of the head—was not forked; there was no sex comb on the right.



Elimination of a crossover maternal chromosome, bearing yellow white and bar explains every feature of the gynandromorph except that the left eye (which was not-bar, and was more completely surrounded by forked bristles than the right eye) was not wine color, but lighter, like the white-wine compound eye of the female region.

Two flies having one wild type X-chromosome and one bearing genes for vermilion, garnet and bar each produced a gynandromorph. Both had been mated to yellow males. No. 14.40a was a gray fly bilaterally divided. On the left, the thorax and abdomen were shorter, and arista, bristles and wing were shorter than on the right; there was a left sex comb. The left eye was garnet bar and the left and median ocelli were lighter than the right. The pigmentation of the abdomen was male on the left and the genitalia were male. On the right, the eye was heterozygous bar, there was no sex comb, and the parts were larger. The absence of vermilion color in the eye of the male region can be accounted for by assuming, either that vermilion though genetically present failed to show, or that the egg had received a crossover chromosome not bearing vermilion. The alternative explanations of elimination of the paternal X-chromosome or derivation from a binucleated egg can be applied.



No. 14.40b was a gray female with a yellow male region including the right half of the thorax and right anterior segment of the abdomen. On the right there was a sex comb, the yellow wing was short, the bristles on the thorax were short and brown, the balancer yellow. Both eyes were heterozygous bar, there was no sex comb on the left gray side where the gray wing and black bristles were long. The

fly is explained by elimination of a maternal X-chromosome, which was a crossover between vermilion and garnet, as shown by F_1 offspring.

g B
———|——— Xm1
———
2

y
——————— Xp   y ——————— Xp

No. 15.8g was an F_1 from a scute female heterozygous for crossveinless vermilion forked due to genes carried in one chromosome and for echinus cut^6 garnet^2 bobbed in the other; she was mated to an X_8 male, a combination of yellow and all of the above characters except scute. The gynandromorph was scute echinus cut^6 vermilion and forked. The presence of two sex combs, of wings of equal length and of female genitalia and pigmentation of abdomen indicated an antero-posterior division into male and female regions. If an egg had received a scute echinus cut^6 vermilion forked-bearing chromosome (which would have been a crossover between cut^6 and vermilion) and the female region consisted of the abdomen, the gynandromorph can be explained by elimination of Xp. The binuclear explanation also applies if at least one nucleus had received a crossover chromosome of the kind indicated by the characters of the male (anterior) region.

[sc ec ct v] f   sc ec ct v f   ?   sc ec ct v f
———|——— Xm1   ———|——— Xm1   ——————— Xm   ———|——— Xm1
———   ———   ———
2   2   2

X_8 X_8
——————— Xp   ——————— Xp .... Yp

No. 113–3 was the offspring of a scute broad eosin female which was heterozygous for echinus ruby tan forked and had been backcrossed to a 7-ple male carrying genes for all of the seven mutant characters. The gynandromorph had a female abdomen and male thorax which was scute and (except for the left humeral patch) forked with broad wings of male length, and two sex combs. The head was divided, for the left eye was eosin, surrounded by not-forked bristles, and the right eye was eosin echinus ruby and the bristles about it forked.

sc br w^e sc br w^e 7-ple
——————— Xm   ——————— Xm   ——————— Xm

7-ple   7-ple   7-ple
——————— Xp   ——————— Xp   ——————— Xp .... Yp

Either elimination of Xm or development from two nuclei affords an explanation.

Twenty-two gynandromorphs have been found in "U-stock," so called because the X-chromosome is somewhat U-shaped; the stock originated from a female that had received a detached chromosome bearing the gene for yellow from a yellow mother having two attached X-chromosomes; the U-shape is not retained if yellow has been crossed out of the chromosome. The stock has been maintained by backcrossing heterozygous females to 7-ple males, which have the sexlinked characters scute broad eosin echinus ruby tan forked.

The next seven gynandromorphs were found in backcrosses to 7-ple or to a similar combination of characters and can be explained by elimination of the yellow-bearing U-shaped X (sometimes carrying one or more of the other mutant genes of the stock as the result of crossing over). The phenotypes were such that they might have been derived from two nuclei, since (occurring in backcrosses) the mutant genes carried by one of the maternal X's were the same as those of the paternal X—hence the male region would be the same in appearance whether it contained a paternal X (after elimination) or had developed from a second egg-nucleus that had received the maternal X that was like the paternal.

No. 113–2 was nearly bilateral. The left side of the head and thorax and abdomen were female with no sex comb and a longer wing. On the left side the eye was red and none of the recessive characters carried by the parents showed. The right side was tan, forked and scute, and was male with a sex comb and shorter wing, which was broad; the right eye and ocellus were eosin, echinus, ruby. The right side thus showed all of the recessive characters that were due to genes carried by the paternal X. The genitalia were male. The fly was mated to a 7-ple female and failed to breed.

y Cl ———————— Xm1   y Cl ———————— Xm1   7-ple ———————— Xm2

7-ple ———————— Xp   7-ple ———————— Xp   7-ple ———————— Xp   .... Yp

No. 113–6 was produced by a female homozygous for echinus and heterozygous for a lethal, and was female except the left side of the thorax. On the left, the thorax, except the humeral patch, was scute and forked; a sex comb was present and the wing, showing the character broad, was shorter than the right. The head was not forked (except one bristle behind the left eye), the eyes were echinus (neither eosin nor ruby), there was no right sex comb, the right longer wing was recorded as "broad," but probably, like those of the mother, was wider than normal only as an effect of echinus. The abdomen was female; the fly was mated, but was injured and it failed to breed.

y Cl ec l ———————— Xm   y Cl ec l ———————— Xm1   7-ple ———————— Xm2

7-ple ———————— Xp   7-ple ———————— Xp   7-ple ———————— Xp   .... Yp

No. 113–7 was a tan fly mostly female (not scute, broad or forked) except around the right eye and on the right side of the head dorsally, where the bristles were forked (a bristle on the right humeral patch was perhaps also forked). There were no sex combs. The right eye and ocellus in the forked region and the ventral part of the left eye were eosin, echinus and ruby, while the dorsal part of the left eye was red and probably not echinus. Since the mutant characters were recessive, the part of the head in which they showed was male.

y Cl t ———————— Xm1   y Cl t ———————— Xm1   7-ple ———————— Xm2

7-ple ———————— Xp   7-ple ———————— Xp   7-ple ———————— Xp   .... Yp

No. 113–8 was a tan fly almost all female with no sex combs and with wings of equal length and female abdomen; it was not scute or broad. More than half of the ventral part of the right eye was eosin, echinus and ruby, and perhaps a little smaller than the other part. The dorsal part of the right eye and all of the left eye were red. The fly was not forked except around the part of the right eye that showed the recessive characters. The diagram for 113–7 applies to this and to the next two flies.

No. 113–9 was similar to No. 113–8. The description is the same except that all of the right eye (save a small red spot at the posterior edge) and the right and median ocelli were eosin, echinus, ruby, with the neighboring parts of the head forked. The left eye was red. The right arista and eye were perhaps smaller than the left.

No. 113–10 was a tan female, with male pigmentation on the left side of the abdomen, where the bristles were forked and the genitalia male; the right side of the abdomen and genitalia were female. The only diagnostic character was forked.

No. 113–1 occurred in a backcross of females heterozygous for U-shape to scute echinus crossveinless cut[6] vermilion garnet forked (X-ple) males; the gynandromorph was entirely wild type female except the posterior part of the abdomen on the right which was male, including the genitalia, and was drawn over to the right side. The male region was probably not yellow and therefore is to be accounted for by elimination of the maternal yellow-bearing U-shaped chromosome. With X-ple substituted for 7-ple the fly is represented by the diagrams for No. 113–2.

No. 113–14 was found in U-stock in a backcross between a tan female heterozygous for 7-ple and a yellow tan male having the curved X-chromosome. The gynandromorph was nearly bilateral on the head and thorax, tan and female on the left and male on the right where it showed the recessive characters of 7-ple due to genes carried by one chromosome of the mother. There was some irregularity in the division, for the right eye, which was eosin echinus and ruby, had a red fleck at the posterior edge, and on the mostly female left side there was a sex comb. Otherwise the left was consistently female with red eye, long arista, not-forked on the head, thorax and second leg and not-scute, and the wing was long and not-broad. The right side had a short arista, a sex comb, a short very broad wing and was scute on the head and thorax. The abdomen had altogether female pigmentation and was not drawn to one side as when divided, but the genitalia were mixed and were male on the right. As the heterozygous mother had been backcrossed to a yellow male of the U-stock the gynandromorph as before can be explained by elimination of the U-shaped chromosome, in this case derived from the father; the binuclear explanation is also possible.

7-ple ——— Xm2	7-ple ——— Xm2	7-ple ——— Xm2	7-ple ——— Xm2
y CI t ——— Xp		y CI t ——— Xp	.... Yp

No. 113–11 occurred in a backcross to 7-ple, but is to be explained by elimination of the X-chromosome (paternal) which was not U-shaped, or else as derived from two nuclei. The head and the left side of the rest of the fly from the mid-dorsal to the mid-ventral line were male and yellow. The aristæ were equal and the bristles on the head were uniform; the eyes were both red. In the left yellow male region, there was a sex comb, the wing was shorter; the abdomen with male pigment on the left was bent toward that side, and the male genitalia were on the side rather than at the tip of the abdomen and faced sideways. On the right thorax and abdomen the fly was tan and female with no sex comb, longer wing and bristles and rudimentary female genitalia. The fly did not show any of the recessive paternal characters.

y CI t ——— Xm1	y CI t ——— Xm1	y CI t ——— Xm1	y CI t ——— Xm1
	7-ple ——— Xp	.... Yp	7-ple ——— Xp

Some gynandromorphs have been found also in outcrosses of females heterozygous or homozygous for yellow and U-shape. The mother of 113–12 carried the genes for yellow and forked in the U-shaped chromosome, and wine (an allelomorph of white) and forked in the other X. She was outcrossed to a yellow-white forked bar male. The gynandromorph was entirely yellow and forked, and had therefore received the U-shaped chromosome from its mother. The head and thorax were bilaterally divided; on the left female side the arista was longer, the eye was red heterozygous bar, the ocellus red, the median ocellus red on the left; there was no sex comb and the wing was long. On the right the arista was short, the eye was white and bar with a red fleck posteriorly; the bristles were shorter, the ocellus white; a sex comb was present and the wing was short. The pigmentation of the abdomen was female but the abdomen was bent to the right; the genitalia were abnormal male on the right and imperfect female on the left. Again the U-shaped chromosome had been eliminated and as it had been received from the mother, the paternal X remained in the male region and the binuclear explanation is not applicable.

y CI f
Xm

y w f B
Xp

y w f B
Xp

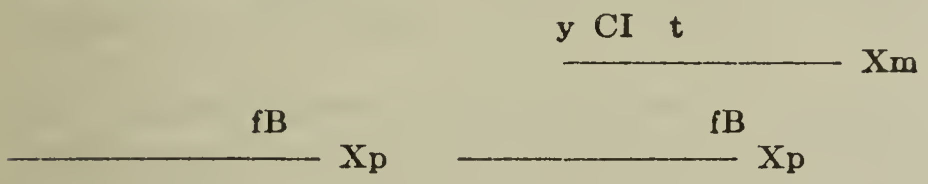
No. 113-23 was a nearly bilateral, wild type color (not yellow) gynandromorph; its mother was homozygous for crossveinless vermilion and forked, and carried also the gene for yellow in a U-shaped chromosome and scute in the other X-chromosome. The father was scute echinus cut^6 garnet^2 bobbed. On the right side of the gynandromorph, the arista was longer than on the left, the eye and ocellus were red, there was no sex comb, the wing was large (female size) and not cut; the right side was not scute or bobbed. The abdomen seemed to be entirely female, but on the left was a little shorter and somewhat abnormal. The genitalia were female. On the left side, the arista was shorter, the eye was echinus and garnet, the left ocellus and also the median were garnet, the bristles on the head and thorax were bobbed (as expected in an XO region). The thorax was a little smaller than on the right, and the wing was smaller and cut; the left sides of the head and thorax were scute. The anterior left leg, however, apparently belonged to the female region, as the bristles on the femur were long and a sex comb was lacking. Since the female region was not-scute, the maternal chromosome containing yellow, which was the curved chromosome, was present, and by its elimination, the gynandromorph had been formed.

y CI cv v f
Xm

sc ec ct^6 g^2 bb
Xp

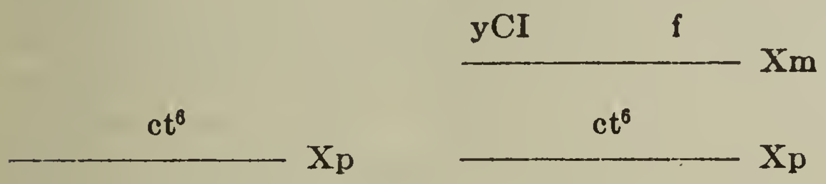
sc ec ct^6 g^2 bb
Xp

Nos. 113–19 and 113–20 were F_1 offspring of females that had a U-shaped X-chromosome carrying genes for yellow and tan, and an X carrying genes for 7-ple, which includes the character forked. They were outcrossed to forked bar males. The head and thorax of No. 113–19 were bilaterally divided, while the abdomen except part of the first two segments on the left was female. The fly was not yellow. The left side of the head and thorax was smaller than the right, with shorter arista, mouth parts, wing and bristles which were forked. A left sex comb was present. The left eye was bar. The right eye was heterozygous bar; the right side was not-forked and no sex comb was present on the right. The fly failed to breed as a female. The right side being not-forked, the maternal chromosome received by the egg was either the entire U-shaped chromosome or a crossover containing the region of the normal allelomorph of forked from the curved chromosome. Owing to peculiarities of crossing over of the curved chromosome, which will be described in another paper, such crossovers are rare; for example, they were only 4.5% of the total in a count of 640 flies. The egg had therefore probably received the maternal U-shaped chromosome and by its elimination the gynandromorph had resulted.



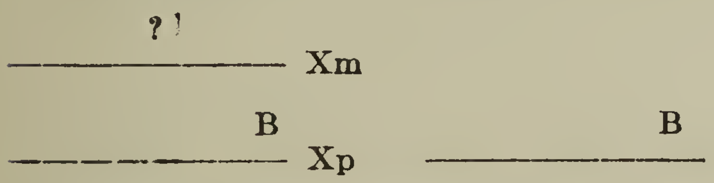
No. 113–20 was male and not-yellow, with a large female head which was not-forked; the eyes were heterozygous bar. There was no sex comb on the right leg, where the bristles were longer than on the left. The thorax was forked, the wings of equal length; a left sex comb was present. The abdomen was male and the fly failed to breed. The probability of elimination of the U-shaped chromosome is the same as for No. 113–19 and the same diagram applies.

No. 113–22 was the offspring of a female carrying genes for yellow and forked in a U-shaped chromosome and forked and bar in the other X-chromosome, mated to a cut^6 male. The gynandromorph was entirely wild type in color, not-yellow, not-forked. The head was female and the abdomen and genitalia were male. The head was large as were also the not-bar eyes. The right side of the thorax was slightly larger than the left, and some of the bristles on the right were longer than the corresponding bristles on the other side; there was no right sex comb and the long right wing was not-cut. The left sex comb, the shorter left cut wing and the male pigmentation of the entire abdomen defined the male region. The only explanation is elimination of the maternal chromosome, which was almost certainly the U-shaped chromosome; there was the same small chance that the maternal X had been a not-U-shaped chromosome from which bar had been crossed out as there had been for a crossover chromosome in the case of Nos. 113–19 and 113–20.



In two gynandromorphs from outcrossed females heterozygous for the U-shaped chromosome, the maternal chromosome was eliminated, as was shown by the characters of the male regions, but there was no means of knowing which of the maternal X's was present in the female region. Origin from two nuclei was not possible. The mothers had genes for yellow echinus and a lethal in the U-shaped chromosome, and the genes for the 7-ple group of characters in the other X. They were crossed out to bar males.

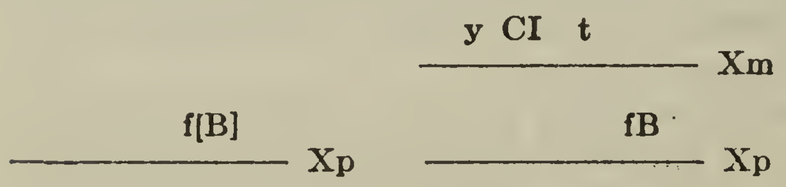
No. 113–5, the gynandromorph offspring of the first, was female on the left side with a heterozygous bar eye, no sex comb, long wing and female abdominal pigment and genitalia. On the right side, the eye was bar, there was a sex comb, shorter wing, and male pigment dorsally on the abdomen which was structurally female. The genitalia were female, partly male on the right side.



In No. 113–4, the second gynandromorph from the same parentage, the abdomen and thorax (with two sex combs) were male and the head female with heterozygous bar eyes. The lack of recessive characters in the male region showed that the maternal X had been eliminated, because both maternal X's carried recessives that would affect a male abdomen. The last diagram applies, except that bar was not diagnostic in the male region, which was judged by the presence of the normal allelomorphs of the characters carried by the maternal X's.

From females having two U-shaped chromosomes both carrying yellow and tan, mated singly or several in a culture to forked bar males, three gynandromorphs have been obtained.

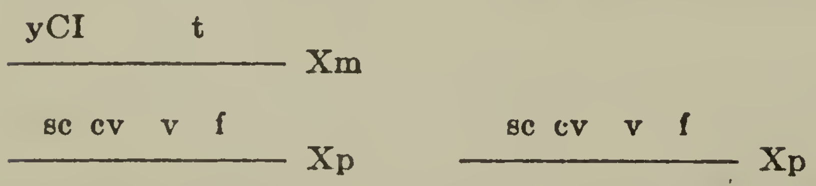
In No. 113–15 the head and thorax were female and the abdomen male. The aristæ were equal and long, the eyes were heterozygous bar, there were no sex combs and the wings were long; the female region was gray and not-forked. The abdomen was male in shape and pigmentation, gray and forked, and the genitalia were male. The gynandromorph can be explained by elimination of a maternal U-shaped chromosome, but not on the binuclear hypothesis.



No. 113–16 occurred in the same mass culture; it had the same constitution but a different regional division. The entire fly was gray (not-yellow). The head was male and forked with short aristæ and bar eyes. The mid-dorsal line of the thorax divided a left male forked region from not-forked on the right; the left wing was short, the right long. Both anterior legs bore sex combs and the thorax ventrally was forked. The abdomen was not-forked and was female in shape and pigmentation except part of the fourth segment on the left and the tip, which were male. The explanation and the diagram for the previous gynandromorph apply also to this, and to the next gynandromorph except that in both of these bar as well as forked was diagnostic in the male regions.

No. 113–17 was female except the right side of the head, the lateral and ventral part of the right side of the thorax (except the humeral patch) and the tip of the abdomen. These parts were male, gray (not-yellow) and forked, with shorter arista, bar eye, sex comb, shorter wing and male genitalia. The more extensive female region (including left head, left and all the dorsal thorax and most of the abdomen) was gray and not-forked, with long bristles and arista, heterozygous bar eye, no sex comb, longer wing, and female pigmentation on most of the abdomen which was of female shape; the male tip of the abdomen was bent slightly to the right.

A female with two curved chromosomes bearing genes for yellow and tan mated to a scute crossveinless vermilion forked male produced a gynandromorph, No. 113–18. The head, first legs with sex combs, and right dorsal thorax (except the humeral patch) were male; these parts were forked, the eyes vermilion, the right side of the scutellum scute, the right wing shorter and crossveinless; the left thorax was not-forked, not-scute and the larger wing not-crossveinless. The abdomen was female with abnormal genitalia. Again the explanation is elimination of a maternal U-shaped chromosome.



The mother of No. 113–21 had two curved X-chromosomes; one of them carried yellow crossveinless vermilion and forked; the other carried yellow echinus cut^6 and garnet^2. She was mated to an X_8 male (yellow echinus crossveinless cut^6 vermilion garnet^2 forked bobbed). The gynandromorph was yellow and female except the right thorax which was yellow and male. It failed to breed. The eyes, both in the female region, were echinus and garnet, the aristæ showed the effect of cut; the head and left thorax were not-forked, not-bobbed. The left wing was longer and cut (not-crossveinless). The abdomen was female. On the right side there was a sex comb and the wing was shorter and both crossveinless and cut, and the bristles on the right thorax were forked and bobbed. The character bobbed showed in the male region in which a Y-chromosome was lacking. The female parts were homozygous for effects of genes carried by the chromosome received from the mother and present also (with other genes) in the paternal X-chromosome, and the male parts showed the characters of the paternal X_8. The fly is explained by elimination of the maternal X.

yCI ec ct^6 g^2
———————————— Xm1

y ec cv ct^6v g^2f bb
———————————— Xp

y[ec] cv ct^6[v g^2]f bb
———————————— Xp

The stock heterozygous for the bent chromosome has produced a gynandromorph, No. 113–13, in which the U-shaped chromosome was certainly not present. The mother, like that of No. 113–12, had a U-shaped chromosome carrying the genes for yellow and forked, and an X-chromosome with genes for wine and forked[5]. The egg received one of the latter. The father was yellow white forked bar. The gynandromorph was forked throughout. All of the left side of the head including half of the mouth parts, and some of the dorsal part of the right side of the head were wild type color; the left arista was longer; the left eye was white-wine compound and heterozygous bar and so was the right eye except a small anterior ventral part which was narrower (bar) and white. The left side of the head and part of the right were accordingly female; both anterior legs were gray with no sex combs; the other left legs were also gray and also the left wing which was deformed. The male region was yellow with brown bristles; it included besides part of the right side of the head, the last two right legs and the right wing and all the abdomen with male pigmentation and genitalia.

w^w f
———————— Xm2

y w fB
———————— Xp

y w fB
———————— Xp

The second gynandromorph described by Duncan (1915) has been understood by Morgan and Bridges to be the offspring of a female heterozygous for cherry club vermilion, and has been interpreted accordingly. It seems more probable from Duncan's statement that the mother was wild type and the father cherry club vermilion. The gynandromorph is then explained by elimination of the maternal-X, as it was quartered and one eye in the male region was cherry (vermilion not showing). The male wing was shorter, not described as club, which is a character that overlaps normal.

3. GYNANDROMORPHS WITH ONE MATERNAL X-CHROMOSOME AND AUTOSOMAL MOSAICS

The "simple cases" of Morgan and Bridges, and a few described by other authors, and the new composites described in section 2 are gynandromorphs whose parents carried no (described) diagnostic autosomal mutant genes. They are of the two kinds of phenotype that can be explained by "simple single elimination," that is, by elimination of a single daughter

TABLE 2—*Analysis of three gynandromorphs that can be explained by single elimination from a regular 2N zygote. The ratios in column 8 indicate the chances for genetic evidence that the not-female region had developed from a separate nucleus (in case of a binuclear origin).*

Kind of parentage	No.	Parentage	Approximate distributions of regions	If derived by elimination		If derived from two nuclei		Type (cf Table 6)	Fertility
				Constitution	Elimination of	Constitution	Chances for genetic evidence		
Group D parental X's all different I Elimination of Xp	438 M. and B. p. 42	w^ev ———Xm1 w ———Xm2 by w^e ———Xp .....Yp	♀; left head and thorax ♂	w w ———Xm2 ———Xm2 w^e ———Xp	Xp	w w ———Xm2 ———Xm2 w^e .....Yp ———Xp	1 : 1	b	$\frac{w}{w^e}$ ♀ +
	7673 M. and B. p. 54 high non-disjunctional strain	w^e ———Xm1 w^ef ———Xm2 .....Ym by B ———Xp .....Yp	♂head left thorax and tip of abdomen on right	w^e(♂)f w^e f ———Xm2 ———Xm2 (.....Ym)? (.....Ym)? [B] ———Xp or w^e(♂)f [w^e] ———Xm2 ———Xm1 [w^e]f ———Xm2 ....Yp .....Yp	Xp or non-disj. +elim. of Xm1	w^e(♂)f [w^e]f? ———Xm2 ———Xm1 or 2 [B] ......Yp ———Xp	0	b	
	A	w^a Xm1 —— vfB ——Xm2 by w^ectvf ———Xp ......Yp	bilateral	w^a w^a ———Xm1 ———Xm1 w^ectvf ———Xp	Xp	w^a w^a ———Xm1 ———Xm1 w^ectvf ......Yp ———Xp	6.5:1	b	

X-chromosome at some division of a developing egg that had received one X-chromosome from the mother. About half of them can also be explained as derived from two nuclei.

ANALYSIS OF SIMPLE GYNANDROMORPHS

Table 2 shows three examples of the analysis that has been made of simple gynandromorphs in order to determine what evidence there is for the two modes of origin.

No. 438, of parentage represented in the third column, is explained by Morgan and Bridges as derived from an egg which received a white bearing chromosome from the mother. The egg was fertilized by X-sperm bearing the gene for eosin. The eye of the male region (left) was mostly white, showing the presence in that region of a maternal X. The eye in the female region was white-eosin compound, as was also a fleck in the left eye. The gynandromorph is explained as the result of elimination of the paternal X at some division. The analysis, based on the characters of the phenotype, was confirmed by the offspring, since the fly bred as a female with one white- and one eosin-bearing chromosome (last column).

Whether the gynandromorph had developed from an egg with one nucleus or with two, the female region would have contained a maternal-X and a paternal-X and would have bred accordingly. If the gynandromorph did in fact develop from two nuclei, one nucleus being of the sort indicated by the characters found in the female region, the chances had been even that the second nucleus would have received the same kind or the different kind of maternal-X, and the chances had been even for its fertilization by X-sperm and Y-sperm. If the second nucleus had received the same kind of Xm as the Xm in the first nucleus (female region), there would have resulted with X-sperm a composite female phenotypically like regular females (table 6, type d), therefore not detectable, and with Y-sperm there would have resulted the gynandromorph that was found (table 6, type b). But if the second nucleus had received the other kind of Xm, there would have resulted either a detectable mosaic female (table 6, type j), or a gynandromorph (table 6, type h) according to which kind of sperm had fertilized the second nucleus. This gynandromorph would have been genetically eosin vermilion in the male region and since vermilion usually does not show in such a case, the gynandromorph would have had an eosin male eye and would be interpreted as derived by elimination of the maternal chromosome. If, therefore, the fly had developed from two nuclei of which one was the nucleus found to be present in the female region there were even chances that a second nucleus could have been demonstrated by direct observation; that is there had been even chances for the ambiguous gynandromorph that was found and for a mosaic female.

As the autosomes were not marked there was no chance of detecting by them the presence of two nuclei and the presence of a (paternal) Y-chromosome in the male region could not be tested by fertility since the sex organs were in the female region.

The data for No. 7673 agree with the explanation of Morgan and Bridges that elimination of the paternal X occurred in an embryo developing from a single nucleus with or without a maternal Y-chromosome. The data in this case, and in many others, are not complete for positive diagnosis, but the gynandromorphs were classified according to the probable explanation, that is by elimination, when there was no positive evidence incompatible with it. For example, in No. 7673 the presence of the paternal X in the female region was not proved by any mutant character since the region did not include either eye, and since bar, the only mutant gene carried by the sperm, could therefore not show (this is indicated by a square bracket); its presence is assumed because the not-forked region being female showed that two X's were present. The head and male left side of the thorax were eosin of the male type and forked. The fly can be explained by elimination of Xp if it is assumed that the maternal-X in the female region was like the one in the male region. The phenotype can also be accounted for if it had developed from two nuclei, but there is nothing to show whether a chromosome like the one in the male region or a different maternal X was in the female region (as indicated in column 7). Because Xm is not diagnostic in the female region and the characters that it bore were not disclosed by offspring, it was not possible to detect a phenotype from a binucleated egg other than the one explicable also by simple single elimination. There was no evidence as to the presence of a Y-chromosome in the male region, since the genital organs were too abnormal for breeding. A third explanation of No. 7673 is possible because its mother was an XXY female. If the fly had developed from an exceptional egg having two maternal X's in one nucleus, fertilized by Y-sperm, the same phenotype would have resulted after elimination of the not-forked bearing (maternal) X; the eye not being included in the female region, the homozygous female type of eosin could not be observed. By this explanation, as by the binuclear, a Y-chromosome is expected in the male region.

The opportunity for diagnosis of No. A (described on page 233) was more favorable because the fly was bilaterally divided and because the sperm of the father carried recessive genes at the same loci as did the two maternal X's. It can be explained by elimination of Xp or on the binuclear hypothesis. If it had developed from two nuclei, one of them containing the chromosomes that were present in the female region, there had been more than even chances that the second nucleus would have produced a composite other than the ambiguous gynandromorph. The maternal X of the female region was shown to be the original apricot-bearing maternal chromosome because the genes carried by the sperm would have disclosed crossing-over at any level. If the male region had developed from a separate nucleus the chances were 1 to 1 that it would have contained the other maternal X for which genetic proof would have been the detectable characters forked bar and not apricot (probably not vermilion for reasons to be referred to later). The chances were 0.85 to 1 for a crossover chromosome carrying genes for apricot forked bar and 0.85 to 1 for the reciprocal crossover. This is based on the recombination percentage of apricot and forked which is 0.46, the chances for recombination being therefore 0.46 to 0.54 or 0.85 to 1. There was also an even chance that X-sperm would have fertilized the second nucleus, resulting in females that would be mosaic if the maternal chromosome were different from that in the first nucleus. This would be the case if the chromosome were the original forked bar bearing chromosome for which the chances were 1 to 1, or if it were either of the reciprocal crossovers between apricot and forked except double crossovers between apricot and vermilion or between vermilion and forked, the chances for the detectable crossovers being 0.59 to 0.41. Therefore, if a second nucleus had been present in the egg with the nucleus that produced the female region, the chances for genetic evidence were 6.5 to 1.

One gynandromorph has been described that is not in accord with either of the alternative explanations and may be referred to here. The female parts of No. 487 are described by Morgan and Bridges (1919, p. 55) as eosin and not miniature, the two eosin eyes (female type) and one wild type wing being included in the female region. The mother was an XXY female homozygous for both eosin and miniature, and the father was wild type, so that if either of the recessive characters showed in an F_1 female region it must be due to the presence of two maternal chromosomes (regular exceptional egg) when the other character is also expected to show since it affected included parts. The explanation by simple single elimination of the wild type paternal X-chromosome in an Xm Xp zygote (with or without Ym), given by the authors, could hold only if the head was male, which is specifically stated to have been not the case. The only formal explanation is somatic mutation of miniature to not miniature, the gynandromorph having been derived from a regular exceptional egg by elimination of the mutated X.

		w^e not-m	
		————————	Xm1
w^e m		w^e m	
————————	Xm2	————————	Xm2
....	Yp	....	Yp

Analyses of other flies will not be given in detail. The conclusions have been based on records of flies enumerated in the footnote,[1] in which page numbers refer to Morgan and Bridges 1919; gynandromorphs not referred to any other author have been described above in section 2.

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[1] Simple gynandromorphs that have been analyzed are:–

Group A (parental X's all alike) Nos. F, p. 39; 29, p. 41; 1808, p. 44; 71, p. 46; 780, Stern 1927.

Group B (from back crosses)

a. egg received the Xm like Xp. Nos. $G_2C_{19}a$, p. 35; 7530, p. 46; X_2, p. 48; 3272, p. 50; N, p. 71; 1768, Mohr 1923; 47, Stern 1927; 76, Stern 1927.

b. egg received the Xm unlike Xp.

I. elimination of Xp. Nos. 477, p. 45; C_2C_{19}, p. 41; X, p. 52; 113–11; 113–14.

II. elimination of Xm. Nos. G_1 ad_2, p. 35; 1–5, p. 36; D, p. 37; T, p. 384; SSO1124 . . . , p. 40; SSO1122 . . . Morgan 1919, p. 384; 2, p. 45; 1819 Mohr 1922; II Gen. Dros.; 113–2; 113–6; 113–7; 113–8; 113–9; 113–10; 113–1; 113–3; 115*a*.

Group C (Xm's alike)

I. elimination of Xp. Nos. X, p. 47; GAb_1 Ca_2, p. 48; 6a; 3(2); 32 /8c; 32 /4a; 23a; 23b; 13b; 31c; 3.83c; III Hyde and Powell 1916; "51" Safir 1920.

II. elimination of Xm. Nos. C_1, p. 6; C_2, p. 7; C_4, p. 8; C_6, p. 9; 3, p. 49; N_2, p. 53; 3(1); 12b; 2; 15b; I and II Duncan 1915; II Hyde and Powell 1916; III Bridges 1913; 1 and 2 Mavor 1924b; 113–15; 113–16; 113–17; 113–18.

III. elimination of Xp and Xm. Hyde and Powell 1916 I.

Group D (parental X's all different)

I. elimination of Xp. Nos. 195, p. 36; 1373, p. 37; 4614, p. 52; 983, p. 56; 2317, p. 49; 7673, p. 54; 5485, p. 55; 1818, p. 38; 438, p. 42; 1813, p. 51; 1333, p. 62; 1713 Mohr, 1923; III Mech. Mend. Her.; I and II Morgan and Bridges, 1913; 5 and No. ? Mavor 1924b; A; 14.40a; 15.8 g.

II. elimination of Xm. Nos. 380, p. 40; 5137, p. 41; 925, p. 43; 5238, p. 45; 941, p. 55; 13a; 12a; Sturtevant 1920; 11.163; 12.176; 14.40b; 113–12; 113–5; 113–4; 113–13; 113–19; 113–20; 113–21; 113–22; 113–23.

EVIDENCE FOR ELIMINATION

The simple gynandromorphs that have been reviewed are classified from the point of view of elimination and the evidence for elimination will first be examined. The flies are placed in four groups according to the ways in which the parental X-chromosomes were marked by mutant genes.

Gynandromorphs from crosses in which the three X-chromosomes of the parents were all different are included in Group D; in Group C are those from crosses in which the maternal X's were alike and were different from the paternal. The gynandromorphs of both groups developed, therefore, from heterozygotes (assuming that the gynandromorphs were derived by elimination). In Group D, 20 flies can be explained by elimination of Xp and 19 by elimination of Xm. In Group C, 13 by elimination of Xp and 20 by elimination of Xm. The total number in the two groups is 33 by elimination of Xp to 39 by elimination of Xm. It will presently be shown that some eliminations probably are not dependent on chance alone; this may have a bearing in relation to 10 flies of Group D II and 4 of Group C II (all elimination of Xm), making the ratio of chance eliminations 33 of Xp to 25 of Xm.

GYNANDROMORPHS FROM BACKCROSSES

Gynandromorphs produced by elimination in backcrosses (Group B) are of especial interest because, in the same experiment, the number of gynandromorphs from homozygotes is expected to equal the number from heterozygotes if the chances of elimination of all chromosomes are equal; that is, two gynandromorphs from homozygotes and therefore not mosaic (fig. 1, a′ and b′) are expected to two mosaics (a and b), one by elimination of X′p, the other by elimination of Xm. The gynandromorphs reported from backcrosses are 8 from homozygotes and 23 from heterozygotes, 5 of the latter by elimination of X′p (fig. 1, b) and 18 by elimination of Xm. Thirteen of the last class and 2 of the one before all contained either a U-shaped chromosome or one bearing the mutation (or deficiency) known as "Notch," and call for further analysis.

GYNANDROMORPHS FROM "U"-STOCK

The frequent elimination of an unusual type of X-chromosome in "U"-stock, a chromosome which is curved in shape, has already been noted in section 2. Gynandromorphs were relatively numerous during the first observations of the stock, four having been found among about 3,300 flies, an occurrence, so far as the counts go, of nearly three times the average number from four stocks examined especially for gynandromorphs (Morgan and Bridges 1919).

These four gynandromorphs have been described in section 2. Two of them (Nos. 113–4, –5) were not completely diagnostic. The other two (Nos. 113–1 and –2 appeared in backcrosses to males not having the U-shaped chromosome and, on examination of these and of five others of similar parentage (Nos. 113–6, –7, –8, –9 and –10) among uncounted flies, it was found that all seven were the result of elimination of the maternal U-shaped X—that is, they were all of type a, fig. 1, and table 3. It seemed probable that in regard to elimination the curved chromosome was not on a par with the other chromosomes with which it had been associated. It has been suggested (L. V. Morgan, 1926), that lagging may be a characteristic of the curved chromosome, which if it occurs at the maturation of the egg might account for the large number of XO males which are regularly found in varying percentages in outcrosses. In recent more extensive counts, a high ratio of non-disjunctional males has again been found, but

TABLE 3—*Frequency of different kinds of gynandromorphs in U-stock and Notch stock compared with the expectation by chance elimination*

A. Gynandromorphs from Backcrosses $\frac{X}{X'}$ x X′Y			
Type of Gynandromorph	a	b	a′ and b′
Elimination of: Chance Expectation	Xm (1)	X′p (1)	X′m or X′p (2)
Found in U-stock:	7 (Xm was U-shaped)	1 (X′p was U-shaped) 1[1] ? (Xm was U-shaped)	0
Found in "Notch" stock:	6 (Xm carried Notch)	0	3
Found in other stocks:	3	3	5
B. Gynandromorphs from Outcrosses			
Zygote	U- or Notch Involved		Not Involved
Elimination of: Chance Expectation from heterozygous females–	U- or Notch-Chromosome (1)	Other Chromosome (1)	Xm or Xp (2)
Found in U-stock:	5	0	1
Found in "Notch" stock:	1	0 or 1[2] ?	2
Elimination of: Chance Expectation from homozygous females	Xm (1)	Xp (1)	....
Found in U-stock:	5	0	....

[1] Possibly derived from a binucleated egg.
[2] Explained by Mohr 1923 as probably a mosaic female by mutation.

not so high a percentage of gynandromorphs. Nevertheless, from the new gynandromorphs there is further evidence of lagging of the curved chromosome. Thirteen additional gynandromorphs were found from various kinds of matings; 11 of them are to be accounted for by elimination of the curved chromosome; in 10 of the 11 (Nos. 113–12, –15, –16, –17, –18, –19, –20, –21, –22, –23) it had been received from the mother; in one (No. 113–14) from the father in a backcross to a male whose X was the U-shaped X. In one gynandromorph (No. 113–13) from a heterozygous outcrossed female the U-shaped chromosome was not involved; it is the only gynandromorph identified as of type a′ or b′ (table 3) that has been seen in the stock. The remaining gynandromorph (No. 113–11) was the only one in which the curved chromosome remained in the male region; since it was the maternal chromosome, the binuclear explanation is applicable; the gynandromorph was unusual in having male genitalia entirely out of the median line, protruding from the left side, and on the other side imperfect parts of female genitalia.

Table 4—*Percentage of patroclinous males from mothers heterozygous and homozygous for a curved X-chromosome*

No.	Parentage	Total No. of Flies	Patroclinous males	
			No.	%
12.4	$\frac{\text{y CI t}}{\text{7-ple}}$ by sccvvf	1244	28	2.25
12.33	$\frac{\text{y CI t}}{\text{ecct}^6\text{g}^2}$ by fB	1894	44	2.32
12.50	$\frac{\text{y CI tf}}{\text{w}^\text{w}\ \text{f}^5}$ by ywfB	6253	252	4.02
12.84	$\frac{\text{yCIecct}^6\text{g}^2}{\text{sccv v}}$ by fB	866	45	5.19
3.33	$\frac{\text{y CI 1 ec}}{\text{7-ple}}$ by $\frac{\text{B}}{\text{fB}}$	635[1]	16	2.52
14.46	$\frac{\text{y CI}}{\text{7-ple}}$ by fB	2013	62	3.08
12.45	$\frac{\text{y CI t}}{\text{y CI t}}$ by fB	1215	139	11.44
12.48	$\frac{\text{y CI t}}{\text{y CI t}}$ by fB	2665	215	8.06

[1] The observed number was 485 and 150 was added for the lethal class of males.

Two of the gynandromorphs first observed among the counted flies (Nos. 113–4 and –5) were F_1's from outcrossed heterozygous females and can be explained as resulting from elimination of a maternal X, but they were not diagnostic as to whether it had been the curved or the other chromosome.

Omitting these two with incomplete data, 20 gynandromorphs have been found in U-stock (see table 3), 18 by elimination of the curved chromosome (whether it had entered in the egg or in the sperm) one in which the U-shaped chromosome was not involved, and one in which the curved chromosome was present but was not the one to be eliminated; a binuclear origin is a possible explanation of the last. The data indicate preferential elimination for the U-chromosome and perhaps notch and chance elimination in other stocks.

If lagging is a characteristic of the curved chromosome a higher percentage of non-disjunctional males is to be expected from females homozygous for the curved X than from females that are heterozygous; this has been found to be the case (see table 4).

GYNANDROMORPHS FROM "NOTCH" STOCK

Mohr (1923) pointed out that gynandromorphs which are "notch" are never notch in the male region. Notch is a deficiency in the X-chromosome, lethal and dominant. Mohr concludes that notch is "absolutely lethal when no unmodified X is present, even when this condition is only established in a small part of the individual while the larger part is of normal viability." That is, if elimination of the paternal X occurs in eggs which receive notch from the mother, the individual dies. Mohr says further "there was no indication of gynandromorphs being more frequent among the offspring of Notch-females than among the offspring of other mutants or of wild type flies. Thus, the deficiency does not favor the occurrence of elimination." But (to carry the analysis further) among the recorded gynandromorphs from miscellaneous experiments with notch stock there is not a sufficient number of gynandromorphs without notch to meet the expectations according to his view; this is not because they have been entirely overlooked or disregarded, for some are recorded. If Xm1 in figure 1 is the chromosome carrying notch, then on Mohr's view b dies and the gynandromorphs to be expected are, 1 notch (in the female region, type a), to 2 not-notch (a′ and b′, not mosaic if X′m2 is like X′p—*i. e.*, in a backcross). If, however, notch does "favor" elimination b will again not be expected (because of the lethal effect of notch), but more notch gynandromorphs (a) will be expected than of either a′ or b′, the ratio depending upon how much the dice are loaded. The gynandromorphs from notch stock that have been explained by elimination in Mohr's two papers (1922 and 1923) are one of each type, possibly, although No. 747 (Mohr 1923) which he says might perhaps be of type b, he considers more probably not a gynandromorph but a mosaic female due to a new mutation.[1] But (omitting Mohr's doubtful case), all the reported gynandromorphs from backcrosses involving notch are 6 of type a, to 2 or 3 (one being perhaps of uncertain parentage) of types a′ and b′ together, whereas to 6 of type a would be expected 12 of a′ and b′ on chance elimination. When gynandromorphs found in outcrosses are included (Mohr No. 1713 in Group D I, No. 941 in Group D II, and C_3 table 5) there are on record 7 of type a to 4 or 5 of a′ and b′ (table 3). The findings are in accord with the results expected if they are due in part to preferential elimination rather than simply to inviability of notch. Embryos with a male notch region would probable be inviable, but on the first view such an elimination would be rare. Mohr noted, however, that there was no evidence for high frequency of elimination, from the numbers of the non-disjunctional males and females (XO and XXY) found in his notch-stock since their number was not greater than that found ordinarily according to Safir's (1920) figures.

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[1] The unexplained character was a purplish color affecting most of one eye. The mother was homozygous for the II-chromosome gene for purple which suggests as another alternative that part of a paternal II-chromosome had been eliminated comprising a short section including the locus for purple (*cf.* summary 11*f*).

There is added evidence for preferential elimination in the case of the U-shaped chromosome in the fact that males (and homozygous females) with the unusual X-chromosome are viable, as was also No. 113–11 in which the curved chromosome was the X of the male region.

The probability of preferential elimination in U- and perhaps to some extent in Notch-stock in connection with a high percentage of gynandromorphs from heterozygotes in general, suggests that lagging may in various degrees sometimes be an effect of some other mutant genes or of other peculiarities of chromosomes. It is possible that environmental factors (e. g., X-rays or temperature) may have a differential effect on the rate of movement of chromosomes as observed effects have indicated for X-rays.

Stern (1927) found a high percentage of primary non-disjunction in bobbed stock, especially from females homozygous for bobbed; one female showed that non-disjunction both of the X- and of the IV-chromosome had occurred. The gynandromorphs that he found in backcrosses were two, both by elimination of a chromosome carrying the gene for bobbed; other gynandromorphs occurred in homozygous matings.

Bridges has described a sex-linked mutant character, minute-n, accompanied by elimination of chromosomes, an effect not limited however to the X-chromosome.

Aside from this stock, there are 136 reported simple gynandromorphs from heterozygotes and 13 to 16 from homozygotes (3 of these were homozygous for bobbed. Gynandromorphs that were derived from outcrosses but were not diagnostic are not recorded in this paper).

Only six or seven of reported gynandromorphs have been in homozygous stocks; this is obviously due in part to the fact that the chief interest in gynandromorphs has depended upon tracing the source of the chromosomes by means of discriminating characters, and in part because homozygous stocks are studied less critically than heterozygous matings. Also not-mosaic gynandromorphs are sometimes less conspicuous than those that are mosaic for mutant characters. The case for gynandromorphs from backcrosses (the other source of homozygotes) has been stated.

Because of the evidence against chance elimination in zygotes heterozygous for the U-shaped chromosome, those gynandromorphs that are known to have contained or may have contained the curved chromosome will not be counted in comparing the number of chance eliminations of Xp and Xm from backcrosses. Gynandromorphs that contained a notch bearing chromosome will also be omitted and, in view of observations on viability to be referred to later, also 3 flies by elimination of Xm (Groups BbII and

DII) which carried lethal genes (one doubtful). There remain from backcrosses 3 by elimination of Xp (Group BbI) and 3 by elimination of Xm (Group BbII) besides 8 from homozygotes (Group Ba) which are not diagnostic for the source of the eliminated chromosome.

Altogether from various matings (Groups B, C and D, omitting cases not by chance elimination) there are 36 gynandromorphs by elimination of Xp and 28 by elimination of Xm, which is approximately the 1:1 ratio that is expected if most of them are due to elimination.

EVIDENCE RELATING TO A POSSIBLE BINUCLEAR ORIGIN OF AMBIGUOUS SIMPLE GYNANDROMORPHS

When both explanations are applicable to simple gynandromorphs it is generally assumed that they were derived by elimination, even if the diagnosis is not complete. For example, No. 7673 has been explained by elimination although the diagnosis is not positive for either X-chromosome of the female region (*cf.* p. 243 and table 2).

Analysis of simple gynandromorphs has shown that in favorable cases if two egg nuclei had contributed to the gynandromorphs of the ambiguous type, there had been even or more than even chances, that one nucleus being of the sort from which the female region developed, the second nucleus would have formed with it a not ambiguous composite. The chances of genetic evidence that a second nucleus had been present in the egg when all the X's of the parents are marked (Group D) depend on whether the characters due to the mutant genes are dominant or homozygous recessive in a female region, on recombination percentages of detectable characters, on whether any lethal genes were present that would affect the ratios of expected composites and on the regional distribution of the fly. Taking these conditions into account, there were found for the ambiguous flies of Group D (*i. e.,* DI) approximately the following chances that there would have been genetic evidence if the flies had developed from two separate nuclei, one of them being the nucleus that gave rise to the female region; for eight flies the chances had been none; for five flies the chances had been 1 to 1; for four 2 to 1; for one 2.7 to 1; for one 5 to 1; for one 6.5 to 1.

If the mothers of composites are homozygous (Group C) there is no evidence discriminating between the two explanations. All of the egg nuclei being alike, no gynandromorph from a binucleated egg will differ from one derived by elimination of the paternal X, and a female derived from two nuclei could not be detected.

In backcrosses (Group B), all gynandromorphs containing non-crossover X-chromosomes can be explained both by elimination and as derived from binucleated eggs. The type that from other matings can be explained only by elimination is the gynandromorph that is heterozygous in the female region and contains the paternal X in the male region. Such a type in a backcross can be explained on the binuclear hypothesis if it is assumed that the X in the male region is not the paternal X but the maternal X that is like the paternal X; and that the two nuclei of the egg had received different kinds of maternal chromosomes. A mosaic female (type j, table 6) is therefore the only not-ambiguous detectable composite derivable from two nuclei without crossing over in backcrosses when the female region is heterozygous. The chances are even for such a mosaic female (table 6, type j) and for one of the two kinds of ambiguous gynandromorphs (table 6, type a or h and type b, a fourth possibility being a phenotype like a female of the regular class, type d). If crossing over would be detectable, the chances of recognizing a binuclear origin are less limited. There have been found in backcrosses 5 gynandromorphs with heterozygous female region explicable as derived from two nuclei containing different kinds of maternal X's (table 6, type h, or type a if they resulted from elimination (Group BbII); if they developed from two nuclei the chances (with the aid of recombinations) for other gynandromorphs and for mosaic females had been for one fly 1 to 1; for two 2 to 1; for one 2.7 to 1; for one 5 to 1.

There have been found in backcrosses 3 gynandromorphs with heterozygous female region explicable as derived from two nuclei containing the same kind of maternal X (table 6, type b, Group Bb1). Two contained in one or the other region a crossover chromosome, but it was not useful in comparing the maternal X's of the two regions because, owing to unfavorable division of the flies, both ends of the maternal chromosome could not be diagnosed in the second region. There had been no chance of detecting a mosaic female with one region like the female part for two of these gynandromorphs and even chances for the third.

When the female region of gynandromorphs from backcrosses is homozygous, there is no discrimination, if they have been derived by elimination, between elimination of Xm and of Xp. Eight such gynandromorphs have been recorded (Group Ba). If they had developed from two nuclei, a second nucleus combined with the homozygote of the female region might have produced a gynandromorph with a different kind of Xm in the male region or a mosaic female containing the different Xm in the second region. The chances of detecting types other than the ambiguous gynandromorph had been for three 1 to 1; for four 2 to 1 and for one 3.3 to 1.

Thus in Groups B and D, there are numerous cases in which if the region that was male had indeed developed from a second nucleus the chances had been even or more than even for genetic evidence for the second nucleus.

Although the probability of elimination in zygotes containing a U-shaped or perhaps a notch bearing chromosome appears to be increased by internal factors favoring elimination it is possible that gynandromorphs containing those chromosomes might develop from two nuclei; the chances of genetic evidence of a separate nucleus were from backcrosses (Groups BbI and II) for eight containing a U-shaped chromosome and for four containing notch 1:1; for one of each kind none; for one containing notch 4:1.

The analysis of simple gynandromorphs from all groups, B, C, and D, indicates that most, if not all of them, were derived by elimination because of the following evidence:

1. The numbers of the gynandromorphs explicable by chance elimination of Xm and Xp are fairly equal (28:36).
2. The gynandromorphs containing a U-shaped chromosome and perhaps also those involving "Notch" appear to have probably resulted from elimination with possibly two exceptions.
3. The ambiguous gynandromorphs give no positive evidence inconsistent with a binuclear origin, but the evidence from all the cases taken together is not simply

negative, because there are a number of cases in which, if a second nucleus had been present in the egg with the nucleus from which the female region developed, the chances had been even, or more than even, that a composite would have resulted demonstrably different from any phenotype resulting after elimination of one of the chromosomes of the female region.

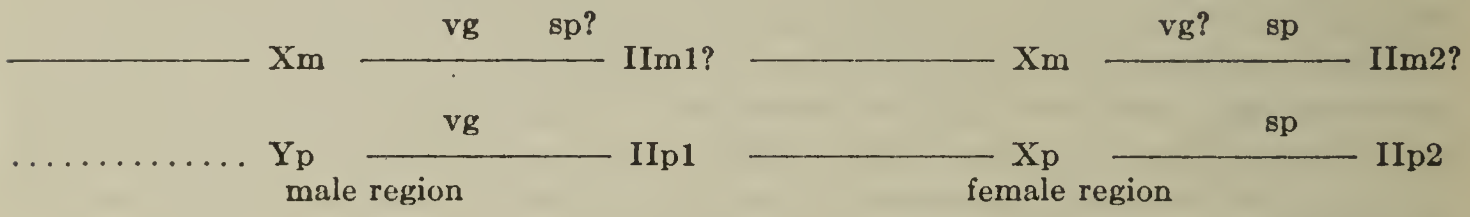
4. Five of the gynandromorphs included among eliminations of Xm were ambiguous because they could be interpreted as derived from two nuclei containing two different Xm's (type h); no other gynandromorphs of the type have been found, and these five all came from backcrosses, the only kind of mating that would produce from eggs with two different maternal X's the same phenotype as one by elimination. From binucleated eggs, gynandromorphs with two different Xm's are to be expected from other parentage as well as from backcrosses and these have not been found containing the original X's of the mother.

This leads to other expectations on the binuclear hypothesis. Unisexual mosaics containing two different original maternal X-chromosomes are to be expected as frequently as gynandromorphs; there are no such mosaic females on record and only one or two mosaic males. One was a doubtful case, perhaps a gynandromorph and there is reason to believe that the other developed from a single non-disjunctional egg. They will be considered later, and also certain other gynandromorphs and mosaics that contained no marked autosomes, but two X's from the mother, not the two original X-chromosomes except when both were contained in the same region (non-disjunction).

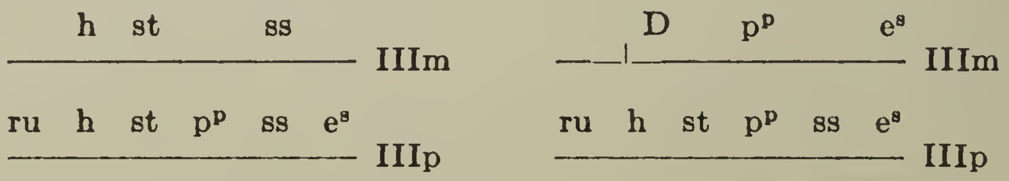
AUTOSOMAL MOSAICS

From miscellaneous experiments there are (as stated) 28 recorded gynandromorphs of type a, probably the result of chance elimination of Xm, a type which is not ambiguous but derivable only by elimination, besides 29 of the same type in which elimination may not have depended on chance alone. It has been roughly estimated that something like equality between the numbers of type a and of autosomal mosaics would be expected if elimination and occurrence of binucleated eggs were of equal frequency provided that there have been as many flies examined with even the simplest autosomal diagnostic markings as there have been in experiments with at least one marked X. It is certain that a very large number of flies have been critically examined in both kinds of experiment, but in the earlier papers no autosomal mosaics have been recorded. Recently, however, 5 composites have been reported, and others have been found, that were mosaic for characters due to mutant genes in the II- or III-chromosome, and therefore referred to but one explanation, that of binuclear origin. At the same time, composites, especially gynandromorphs, explained by simple single elimination, are not as frequently reported when they are found as formerly.

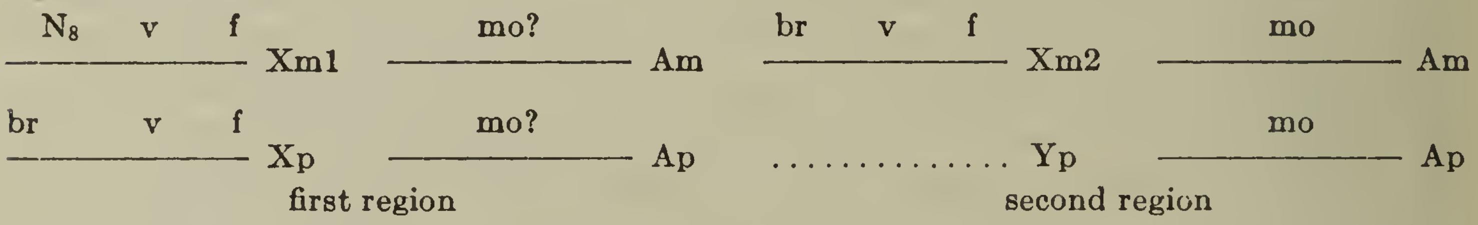
One of two autosomal mosaics described in "The Genetics of Drosophila" (1925), was a gynandromorph mosaic for vestigial and speck, two recessive characters due to genes that were carried each in one II-chromosome of each parent: that is, the parents were F_1 hybrids of two recessive strains (No. V, G. D., table 6, type c). The maternal II-chromosomes of the two pronuclei were different (unless both were crossovers between vestigial and speck) and certainly they had been fertilized by different kinds of sperm. The sperm that took part in the male region carried the gene for vestigial and the Y-chromosome, and the other sperm carried speck and the X-chromosome. In the gynandromorph then there were two different II-chromosomes from the father and probably also two different ones from the mother—in either case a binuclear situation.



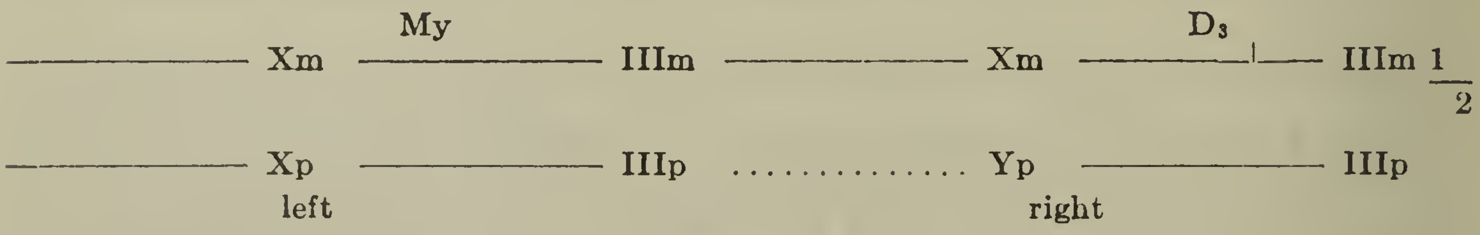
The other mosaic (No. IV, G. D., table 6, type e) was a female and had in the two regions one original and one crossover III-chromosome from the mother. The father was homozygous for all the recessive genes carried by the mother and the constitution of the mosaic is represented in the following diagram.



Mohr (1923) found a notch gynandromorph (No. 2134, table 6, type i) mosaic for an autosomal modifier of vermilion which was present in the stock. The gynandromorph was "quartered," the right anterior and left posterior parts were female. It was pure vermilion and the male eye was of the color due to the modifier when homozygous, whereas the female eye was not modified. The two regions were therefore different in autosomal constitution and had presumably developed from two different zygotes in the egg.

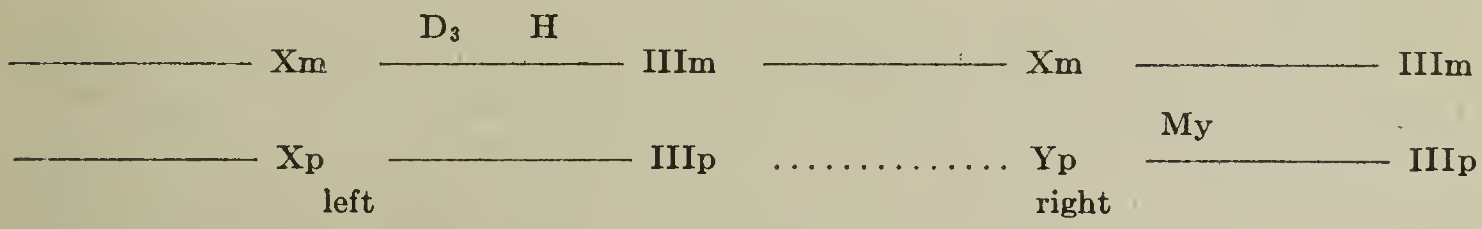


Stern has found a gynandromorph autosomal mosaic (No. 1734, unpublished, *cf.* table 6, type c) in a cross between a wild type male and a female carrying minute-y in one of the third chromosomes and dichæte 3 and hairless in the other, all dominant characters. The left half of the thorax and abdomen except the tip was female and minute-y; the corresponding parts on the right were male and dichæte, the genitalia male. The head bristles were equal on both sides, probably minute; the right eye and arista were abnormally large.



The phenotype is explained if the nucleus from which the left side developed had received a chromosome bearing minute-y from the mother and had been fertilized by X-sperm, and if the nucleus of the right side had received a crossover III-chromosome bearing dichæte but not hairless and had been fertilized by Y-sperm.

A second autosomal mosaic found by Stern (No. 1844, unpublished, *cf.* table 6, type c) was also a gynandromorph. The mother was dichæte hairless (heterozygous for both dominant characters for which the genes were carried in the same III-chromosome); the father was "minute-y" (also heterozygous and dominant). The left side of the head and thorax was dichæte hairless, not-minute, with no sex comb; the right side where a sex comb was present was not-dichæte not-hairless but minute-y. The abdomen was male type but curved to the right. Evidently the composite had received different III-chromosomes from both parents, and the nuclei had been fertilized by X-sperm and Y-sperm.



It may be noted that the explanation of the autosomal mosaics by elimination of an autosome, even if it were not excluded on the ground of inviability, would not apply to any of the autosomal mosaics except perhaps to Mohr's.

The autosomal mosaics are exactly comparable to Toyama's two mosaics of the silkworm moth and are of the type that Morgan and Bridges foretold should be found if binucleated eggs occur in *Drosophila* as Doncaster had observed in Abraxas.

REGIONAL DIVISION OF COMPOSITES

The regional division of composites does not supply a criterion as to their origin. There is no distinctive difference in regard to regional division between the descriptions of autosomal mosaics (from two nuclei) and of some gynandromorphs undoubtedly derived by elimination. The autosomal mosaics that are reported were all bilateral in part, but in two all of the abdomen was included in one region, in one all of the head. It is generally supposed that when gynandromorphs by elimination are bilateral the elimination has taken place at an early division. Gynandromorphs that are very unevenly divided and are mainly female are among the probable eliminations. Some gynandromorphs of an ambiguous phenotype are more male than female. Three extreme examples are flies with only half or three fourths of the abdomen female (No. X_2, p. 48, Morgan and Bridges 1919; No. 377, Stern 1927; No. 21, table 5). Morgan and Bridges have suggested somatic non-disjunction as an alternative explanation for No. X_2. On this view the fly started as a male. "Somatic non-disjunction resulted in a cell with both daughter X's present, and this gave rise to the female parts." In such a case the sister cell containing a Y- but not an X-chromosome "would probably die." Stern thought that a preponderance of male parts was more likely to occur if the fly had developed from two nuclei than by elimination and there were other reasons as will appear for the first explanation in the case of Stern's gynandromorph No. 377 (*cf.* p. 273). Some gynandromorphs, however, that are not explicable as having developed from two nuclei are more male than female. For example, all of the abdomen as well as half the head and thorax of No. 3 (Morgan and Bridges 1919, p. 49) was male and the gynandromorph can be explained only by elimination because the male region contained the paternal X-chromosome.[1] Genitalia are sometimes mixed, and Sturtevant (1921a) suggested, in the case of a gynandromorph of *D. simulans,* that the presence of extra parts might perhaps indicate an origin from two nuclei. In one fly (113–11, p. 237) male genitalia and imperfect female genitalia were not only present, but were separated, the male parts protruding laterally from the left side, and the female lying to the right of the tip of the abdomen. In this case there were some other considerations unfavorable to the explanation by elimination.

Although miscellaneous experiments do not furnish numerical data for the frequency of binucleated eggs, still they show that binucleated eggs are of rare occurrence even compared with the infrequent phenomenon of elimination of a sex chromosome. The chances of detecting composites from two nuclei were increased in the following experiment, and it afforded a direct comparison with frequency of elimination.

4. COMPOSITES WITH MARKED SEX CHROMOSOMES AND AUTOSOMES

The aim in preparing the stocks for detecting composites was to mark the chromosomes in such a way that any two combinations of different chromosomes from a given pair of flies would differ in some conspicuous character, preferably also one of general distribution and good viability.

EXPECTED RATIOS FROM BINUCLEATED EGGS CONTAINING MARKED X-, II- AND III-CHROMOSOMES

The expected ratios from two separately fertilized nuclei from parents with two different kinds of X-chromosomes, in an autosomal backcross, have already been referred to (p. 228). With more discriminating marks the expected number of mosaics derivable only from two nuclei rapidly becomes greater in comparison with the number of gynandromorphs of the ambiguous phenotype derivable also by elimination. If the maternal X's are not only different from the paternal X, but also different from each other, the four nuclei to be expected from the two kinds of egg pronuclei fertilized by X- and Y-sperm, if associated by chance in pairs in one egg, would give 2 gynandromorphs with maternal X's that were alike in the male and female regions (type b), to 2 gynandromorphs with unlike maternal X's (type h) to 1 female and 1 male that would be mosaic because the maternal X's of the two regions were different (types j, l) to 1 female and 1 male

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[1] As this paper goes to press, a gynandromorph has been found that is entirely male except the left eye and surrounding bristles which were larger than on the right side. The left eye showed the mutant characters to be expected in a female. The characters of the extensive male region were those due to the paternal-X, and elimination of the maternal chromosome affords the only explanation.

(types d, f) that would be like the females and males of the regular classes and would therefore not be detectable (cf. Table 6).

If the II-autosomes were also marked so that there would be four possible kinds of fertilized nuclei different for II-autosomal characters, then the possible combinations of these nuclei in a binuclear egg are 4 like pairs to 12 unlike; and composites developing from them would be 1 not-mosaic to 3 mosaics for II-chromosomal characters. If the third chromosomes were also marked, there would be within each of the last classes 1 not-mosaic to 3 mosaic for III-chromosome characters, or altogether, 1 not-mosaic to 15 mosaic for some autosomal character. That is, if the chromosomes of the parents are marked, as in fact they were in part of the experiment, namely in such a way that no two combinations of X, Y, II and III in a fertilized nucleus would be the same, the expectation for composites from two nuclei is: 1 not autosomal mosaic to 15 autosomal mosaics of each of the classes expected on the basis of sex-linked characters (table 6).[1] One of these phenotypes and only one would also result from elimination, namely, the gynandromorph that contained only one kind of maternal X-chromosome and that was not-mosaic for autosomal characters (type b). The expected ratios of detectable composites from binucleated eggs are then (table 6): 2 gynandromorphs of the ambiguous type b to 1 male, 1 female (types j and l) and 2 gynandromorphs (type h) that contain two different maternal X-chromosomes, to 120 autosomal mosaics (whether gynandromorph or unisexual). To this number of detectable composites there would be 2 flies (1 male and 1 female, types d and f) indistinguishable from the regular classes. Thus, 98.5% of all flies derived from two nuclei would be mosaic and 98.4% of these could not be explained by elimination, so that 97% of all the flies that developed from two nuclei would give genetic evidence of their origin.

The characters used in the experiment were, in the first or X-chromosome: eosin (w^e), a pale reddish-yellow eye color; cut (ct), affecting mainly the margin of the wing; vermilion (v), a brilliant red eye color resulting in a color almost white when in combination with eosin; forked bristles (f); the dominant character bar (B) or narrow eye; and in the paternal X, yellow (y) body color. In the second chromosome: the dominant star (S), a roughness of the eye, and black (b) body color. In the third chromosome: the dominant characters dichæte (D), shown by spread wings and loss of some thoracic bristles, and hairless (H), distinguished by loss of some bristles on the head and short 5th vein of the wings. The arrangement of markings was changed at one point in the experiment, and in some of the matings the entire set of characters was not present, with the result that the parentage of all of the gynandromorphs was not identical. The most completely marked parents were heterozygous bar, heterozygous black, hairless females mated to yellow star black dichæte males, with the distribution of mutant characters indicated in the diagram.

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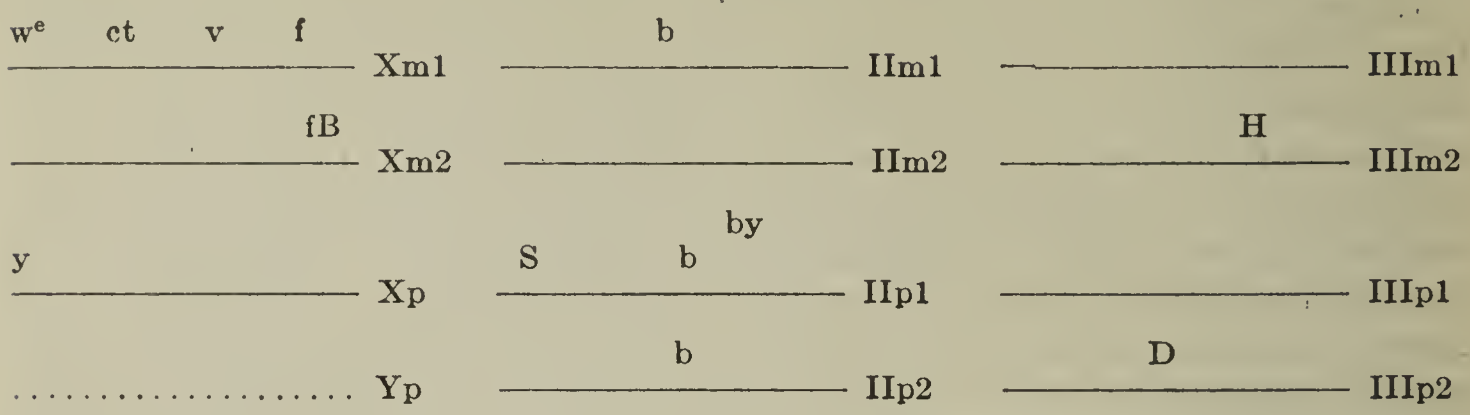
[1] From a female with three pairs of marked chromosomes there are 8 different kinds of nuclei; after fertilization by 8 different kinds of sperm there are 64 different kinds of zygotes. The number of possible pairs of these zygotes (in a binucleated egg) is 64 × 64, among which there are $\frac{64}{2}$ (1+64) or 2,080 different kinds of pairs; 64 of them are pairs of two nuclei that are alike and would produce males and females like the regular classes of flies; they occur in the proportion of 1 of each to 2 of each of the other kinds of pairs which are composed of unlike nuclei, and of which there are altogether 4,032. The different types of gynandromorphs and mosaics are expected in the proportions stated in the text (*cf.* also table 6).



Such matings admitted of the number of combinations of nuclei of the theoretical calculation. They were used in more than two thirds of the experiment, except for a few matings when star was not present. In the rest of the experiment, the autosomes of the sperm were not differentiated (as shown in the parentage of No. 1, table 5), but the markings admitted of detectable recombinations of characters by crossingover in the two maternal pairs of autosomes so that there was the same number of possible different kinds of nuclei. But whereas with the markings shown in the diagram the chances were even for every kind of nucleus, the frequency of occurrence of some of the nuclei from the second kind of mating depended on the recombination values of dichæte hairless and of star back. The outcome is however nearly the same. In the first case, the chances among flies from two nuclei were 62 chances for a phenotype giving genetic evidence of separate nuclei to 1 chance for the ambiguous phenotype, and in the second case, the chances were 58 to 1.

It may be stated at the outset that, in spite of all the chances of recognizing the binuclear origin of a fly in case any had been produced from two nuclei, no such composites were found among 74,219 counted flies from final matings.

COMPOSITES FOUND IN THE EXPERIMENT

The composites that were found were a group of non-disjunctional mosaic females, and 13 gynandromorphs, one of the latter in a preliminary stock leading to the final matings, and twelve among the 74,219 counted flies. The mosaic females will be described in sections 5 and 8. (Mosaics probably due to somatic mutation will not be described.)

The value of the characters for the diagnosis of the gynandromorphs depends on the regional division of each individual and will be considered after giving descriptions of the flies. Variations in parentage and diagrams representing the constitution of the flies are shown in table 5.

No. 1 was a fly of wild type color. The head was largely female, but partly male for the right eye was eosin except for a small red fleck and the region around it was forked. The thorax was female with equal not-cut, dichæte hairless wings and no sex combs and was not-forked. The abdomen was male except that a small part dorsally at the posterior end on the left was female. The surfaces of the eyes were alike, probably not-star, and the fly was uniformly not-black. The characters dichæte and hairless were not diagnostic, because there was no regional division of

	p. 7	—— Xm1 —— —— N —— Xm2 —— —— by S D —— Xp —— —— .... Yp —— ——	bilateral with ♂ genitalia	w^e w^e —— Xm1 —— Xm1 —— Xp $+^S$ D $+^S$ D	Xp	13:1	
	C_5 p. 7	C_{II} l c —— II m1 b vg —— II m2 by b pr cpx sp —— II p1 b prc px sp —— II p2	bilateral	b vg b vg ——II m1 ——IIm1 b pr c px sp b pr c px sp —— II p —— IIp b b	X?	2:1	♂ –
	77 p. 35	w^e cr —— Xm1 —— w^e —— Xm2 —— by w^e cr —— Xp —— .... Yp ——	bilateral	w^e —— Xm w^e —— X? w^e —— Xp cr cr	X?	6:1	

TABLE 5—*Composites with Marked X's and Autosomes.* (*not from binucleated eggs*)

Kind of composite	No.	Parentage			Approximate regional distribution	Constitution		Elimination of:	Chances of detecting a binuclear origin	Fertility
		X and Y	II	III						
Gynandromorph	1	$\underline{w^e\ ct^6\ f}$ Xm1 $\underline{fB}$ Xm2 $\underline{y}$ Xp .... Yp	—— $\underline{S\quad b}$ by $\underline{b}$ $\underline{b}$	$\underline{D\ H}$ —— —— ——	♀; part of head and most of abdomen ♂	$\underline{w^e\ ct\ f}$ Xm1 —— Xp $+^S\ ?\ \frac{b}{+}\ DH$	$\underline{w^e\ [ct]\ f}$ Xml $+^S\ ?\ \frac{b}{+}\ [DH]$	Xp	11:1	
	4		as above		♀; left thorax and left anterior abdomen ♂	$\frac{y}{[S]bDH}$ Xp	$\underline{fB}$ Xm2 $\frac{y}{+^Sb\ \ DH}$ Xp	Xm		
	5		as above		♂; head and left thorax ♀	$\underline{w^e\ ct\ \vert\ f\ B}$ Xm½ $\underline{y}$ Xp $S\ ?\ \frac{b}{+}+^D H$	$\underline{[w^e]\ ct\ \vert\ f\ [B]}$ Xm½ $[S]\ \frac{b}{+}+^D H$	Xp	30:1	♂ −
	6		as above		♀; left head, rt. thorax (exc. humeral patch) and rt. abdomen ant'ly ♂	$\underline{w^e\ ct\ f}$ Xm1 $\underline{y}$ Xp $+^S\frac{b}{+}+^D+^H$	 $\underline{y}$ Xp $+^S\frac{b}{+}\ ?+^D+^H$	Xm		$\frac{w^e\ ct\ f}{y}$ ♀ +
	14	$\underline{w^e\ ct\ vf}$ Xm1 $\underline{fB}$ Xm2 $\underline{y}$ Xp .... Yp	$\underline{b}$ —— by $\underline{S\ b}$ $\underline{b}$	—— $\underline{H}$ $\underline{D}$ ——	♀; two posterior abdominal segments and genitalia on left ♂	$\frac{y}{[Sb\ D\ H]}$ Xp	$\underline{fB}$ Xm2 —— Xp $+^S\frac{b}{+}D+^H$	Xm		
	15		as above		nearly bilateral; more ♀ anteriorly; last segment and genitalia ♂	$\underline{w^e\ ct\ vf}$ Xml $\underline{y}$ Xp $S\frac{b}{+}D+^H$	$\underline{[w^e]ct[v]f}$ Xml $[S]\frac{b}{+}\ D+^H$	Xp	14:1	♂ −
	18		as above		♀; ventral thorax and part of left eye ♂	$\underline{w^e[ct]v^*f}$ Xml $+^Sb[DH]$	$\underline{w^ectvf}$ Xml $\frac{y}{+^SbDH}$ Xp	Xp	14:1	♀ −
	21	$\underline{w^e\ ct\ vf}$ Xml $\underline{fB}$ Xm2 $\underline{y}$ Xp .... Yp	$\underline{b}$ —— by $\underline{b}$ $\underline{b}$	—— $\underline{H}$ —— $\underline{D}$	♂; abdomen partly ♀	$\underline{[fB]}$ Xm2 $\frac{y}{b[DH]}$ Xp	$\underline{fB}$ Xm2 $bD+^H$	Xp	2:1	♂ −
	22		as above		♀; rt. head and thorax ♂	$\underline{w^ectvf}$ Xml $\underline{y}$ Xp $\frac{b}{+}DH$	$\underline{w^e[ct]v^*f}$ Xml $\frac{b}{+}[D]H$	Xp	14:1	
	17	—— Xml $\underline{fB}$ Xm2 $\underline{w^e\ ct\ vf}$ Xp .... Yp	—— —— by $\underline{b}$ $\underline{b}$	—— $\underline{H}$ —— ——	♀; left head and left ventral thorax ♂	$\underline{w^e[ct]v^*f}$ Xp $\frac{b}{+}H$	—— Xml —— Xp $\frac{b}{+}H$	Xm		♀ −
	C_3 p. 7	$\underline{w^e}$ Xml $\underline{N}$ Xm2 —— Xp .... Yp	—— —— by $\underline{S}$ ——	—— —— $\underline{D}$ ——	bilateral with ♂ genitalia	$\underline{w^e}$ Xml —— Xp $+^S D$	$\underline{w^e}$ Xml $+^S D$	Xp	13:1	
	C_5 p. 7		$\underline{C_{II}\ \ l\ \ c}$ II m1 $\underline{b\ \ vg}$ II m2 by $\underline{b\ pr\ cpx\ sp}$ II p1 $\underline{b\ prc\ px\ sp}$ II p2		bilateral	$\underline{b\quad vg}$ II m1 $\frac{b\ pr\ c\ px\ sp}{b}$ II p	$\underline{b\quad vg}$ IIm1 $\frac{b\ pr\ c\ px\ sp}{b}$ IIp	X?	2:1	♂ −
	77 p. 35	$\underline{w^e}$ Xml $\underline{w^e}$ Xm2 $\underline{w^e}$ Xp .... Yp	$\underline{cr}$ —— by $\underline{cr}$ ——		bilateral	$\underline{w^e}$ Xm $\frac{w^e}{cr}$ Xp	$\underline{w^e}$ X? cr	X?	6:1	

parts affected by them. (There was a slight chance that the fly was an F_2 or the offspring of a backcross from one of the parents, column 1, but it was almost without doubt an F_1.) Elimination of Xp is an adequate explanation, and the binuclear explanation is also possible.

No. 4 was mostly black female, but partly yellow black male on the left. The female region included the head, right thorax and most of the abdomen; the eyes were heterozygous bar and not-star, there was no right sex comb, the right wing was dichæte hairless. The male region included the left side of the thorax with shorter bristles, a sex comb and shorter dichæte hairless wing, and the left anterior part of the abdomen, which was shorter than the right. Elimination of the maternal X explains the gynandromorph and Xp being the chromosome of the male region there is no question of two nuclei.

No. 5 was a heterozygous black fly with the head and left side of the thorax female, not-forked, with no sex comb and not-cut wing. The right thorax was forked; a sex comb was present on the right leg, the right wing was cut. The abdomen was male. Both eyes were in the not-forked female region and were heterozygous bar not favorable for determining star. The wings (one in each region) were both hairless and not-dichæte. The fly failed to breed as a male. No. 5 can be explained by elimination if the egg had received a crossover chromosome that carried genes for cut forked and bar, probably also for eosin, and if the paternal-X had been eliminated. The binuclear explanation also applies.

No. 6 bred as a heterozygous black female, having genes for eosin cut forked in one X and for yellow in the other. A line dividing the female from the male region left a yellow male half of the head with red eye on the left and a yellow male half of the thorax and three anterior segments of the abdomen on the right; the male left side of the head and somewhat short male right side of the thorax had short brown bristles, except the humeral patch, which was like the female region. There was a sex comb and a shorter yellow wing on the right. The red-eyed gray (heterozygous black) female region had long black bristles. There was no left sex comb and the left wing was long. Xp being the chromosome of the male region, elimination of Xm is the only explanation. Autosomal characters, not-star, not-dichæte and not-hairless were alike in both regions, as would be expected. The yellow region was noted as yellow black, being probably darker than yellow owing to heterozygous black.

No. 14 was nearly female throughout, not-black, with heterozygous bar eyes and with no sex combs, but the posterior end of the abdomen on the left side was yellow and somewhat shorter than the right with shorter lighter bristles. The genitalia were male on the left and partly female on the right. The male region included no parts affected by the autosomal mutant genes of the parents. The fly can be explained only by elimination of the maternal-X.

No. 15 was a nearly bilateral gynandromorph, neither yellow nor black. Both eyes were red and star, not-bar. Most of the head, the left thorax the ventral right side of the thorax and almost all of the left side of the abdomen were female, not-forked; there were no sex combs, the left wing was not-cut. The male region included a small posterior part of the head on the right, the right dorsal thorax, the right side of the abdomen dorsally and ventrally, and all of the last abdominal segments; in these parts which were forked the bristles were shorter. There was no right sex comb (the thorax was not male ventrally), but the right wing was shorter and cut, and the abdomen shorter than on the right side. The pigment on the right side of the abdomen and on the posterior part of the left side was of male pattern and the genitalia were male. Both male and female regions were not black, dichæte and not hairless. Star was not diagnostic as both eyes were in the female region. If the egg had received one of the maternal X's that carried eosin cut vermilion forked, elimination of the paternal-X explains the phenotype. The binuclear hypothesis is also consistent.

No. 18 was mostly female, but some recessive characters indicated male parts. The head showed a recessive sex-linked character in that the ventral part of the left eye was overlaid with eosin (but not vermilion); the bristles near the eosin part were lacking so that the character forked could not be checked. The sides and under surface of the thorax were forked. There were no sex combs. The wings were of equal length and were not-cut, though the outer edge of the right wing was irregular. The fifth abdominal segment was irregular on the left. The fly was entirely black not-star. Dichæte and hairless were present in the female region but did not affect any parts in the male region. The phenotype is the one common to both exlanations, but the regional division was not to be expected on the binuclear hypothesis.

No. 21 was a black fly nearly all male. The female region included only the larger part of the abdomen; the pigmentation on the last segment and on the left of one and a half segments anterior to the last was male (the description of only the dorsal side is recorded). The eyes were bar, the head and thorax forked, dichæte and not hairless. Elimination of the paternal X or development from a binucleated egg are both consistent explanations.

No. 22 was not-black, mostly female; the smaller right side of the head and part of the right thorax were male as shown by the shorter arista and small bristles. The left female eye was red, the right was eosin (not vermilion), and the male region was forked. The not-cut wings, not-forked scutellum, and legs without sex combs belonged to the female region. Dichæte affected only the female region; both male and female sides of the head showed the character hairless. Like the two preceding gynandromorphs, it was of the ambiguous phenotype.

No. 17 was a gynandromorph found in a preliminary stock and not among the counted flies. It was female except for the male left side of the head and ventral thorax and left humeral patch, a region with shorter arista and bristles, which were forked, and a sex comb. The left eye and ocellus were eosin (not vermilion). The right eye, in the female region, was red, and all the region was not-forked. It included the not-cut wings. The head on both sides and the wings were hairless. The gynandromorph can be explained only by elimination of the maternal X.

In connection with the marked gynandromorphs the third of the critical cases of Morgan and Bridges (C3, *see* table 5) is of interest because characters in both large autosomes of the father were useful in analysis of the gynandromorph. Their fifth critical case (C5) was from a backcross for black, and No. 77 was from parents heterozygous for an autosomal modifier of eosin. In neither of these cases is it known whether the maternal X was the one present in the male region. If the gynandromorphs had developed from two nuclei, autosomal characters were diagnostic. All three flies are of the phenotype to which both explanations apply.

RATING OF THE GYNANDROMORPHS

The chances that, with the given zygote in the female region, a second nucleus if it had been present in the other region would have been detected, have been calculated for each ambiguous phenotype from the data in table 5.

In No. 1 the X-chromosome of the male region was the maternal X, bearing eosin and forked, and presumably cut, although the character did not show because both wings belonged to the female region. Explaining the fly by elimination, the same X is assumed to have been the one present in the female region.

In diagnosing the constitution in case the fly developed from two nuclei, dichæte and hairless were useless owing to the division of regions. Both eyes were slightly rough, rated as not-star, and at any rate were alike; both regions were not-black—hence the two regions were alike for diagnostic autosomal characters, and also neither one was bar. So far as diagnosis was possible, the fly belonged to type b (table 6).

If the fly had developed from two nuclei, one of them being of the kind that formed the female region, the chances were 11 to 1 that the second nucleus would have been of a kind to produce some mosaic other than the ambiguous gynandromorph. The chances were 1 to 1 that the second nucleus would have been fertilized by X-sperm; the chances were 1 to 1 that the maternal X-chromosome bearing bar would have been the chromosome of the male region (chances of crossing over in the X-chromosome did not add anything because there were no offspring to disclose the recessive characters in the female region and they could be assumed to be the same as those in the male region, conformable to the requirements for elimination); the chances were 1 to 1 that the maternal II-chromosome would have been the one bearing star and black, and .6 to 1 for a recombination of star and black. One of the composites for which the chances were 1 to 1 would have been like the regular female, leaving 10.9 chances to 1 that a second nucleus could have been positively diagnosed.

In No. 5 the eyes were both in the female region and bar and star were therefore not diagnostic, and as there were no offspring the recessive characters in the X-chromosomes were also useless. There were even chances for X-sperm, and for black. The female region was dichæte and not hairless due to a recombination for which the chances are 0.33 to one chance for an original chromosome; the chances were therefore 3 to 1 for each of the original chromosomes to have occurred in a second nucleus and 1 to 1 for the reciprocal crossover. Computed on this basis, the chances of detecting a second nucleus had been 30 to 1.

Calculations were similarly made for the other gynandromorphs found in the experiment, and for three other gynandromorphs with marked autosomes (C_3, C_5 and No. 77) previously described by Morgan and Bridges (*cf.* table 5). The ratios are: 2 to 1 for No. 21 and C_5; 6 to 1 for No. 77; 11 to 1 for No. 1; 13 to 1 for No. C_3; 14 to 1 for Nos. 15, 18 and 22; 30 to 1 for No. 5. The figures show that the chances had been in favor of genetic evidence for the binuclear origin if the composites had been derived from two separate nuclei, while in fact the only phenotype that was found was the one in common with elimination.

There is the following evidence that the 12 gynandromorphs which were found in the experiment and one in a parent stock had probably been derived by elimination (types a and b). Proof that 4 of them were so formed is the presence of the paternal chromosome in the male region (type a). The phenotypes of 6 others having a maternal X in the male region (type b) might have been derived from two nuclei, but it is probable that they had not so developed because (1) on chance elimination about as many gynandromorphs are expected by elimination to be of type b as of type a, and the number observed agrees with the expectation as far as it has any significance; (2) although in each of the ambiguous cases (type b) the unfavorable division of the composites and absence of offspring would not have permitted diagnosis of all of the possible phenotypes from two nuclei, still a large number of possible types other than b were by positive diagnosis excluded; (3) about 97% of possible composites of binuclear origin could have been positively diagnosed as such, and no composites were found that admitted of the binuclear explanation except the one type of gynandromorph that can be explained also by simple single elimination. Three gynandromorphs that were not diagnostic probably resulted from elimination because the regional division was extremely unequal and they were mostly female.

The binuclear hypothesis is in some cases certainly and in the other cases probably inapplicable to the non-disjunctional mosaic females which were found in the experiment, and they will be described among the classes referable to single or multiple elimination.

CONCLUSION FROM THE EXPERIMENT

Within the conditions of an experiment that produced 12 gynandromorphs probably by simple elimination, there were no composites that could be positively diagnosed as derived from two nuclei, although if any had occurred, about 97% of all types of composites from two nuclei were expected to be not of the ambiguous type, and to be detectable if favorably divided, and the chances were 62 to 1 in favor of a not-ambiguous detectable type. On the theory of probability, if composites from binucleated eggs occur on an average as often as 1 in 74,000 flies, it is to be expected that there would fail to be any in one out of three counts of 74,000 flies each; but if they occur as often as 10 in 74,000, the chances are that only one in 20,000 such counts would fail to show any. It will be shown that the mosaic females found in the experiment may have been produced by single or multiple elimination but not from binucleated eggs.

An environmental factor is known which increases the frequency of elimination. Mavor (1924*a*) has shown that non-disjunctional males and females are more numerous from females X-rayed before fertilization (before or soon after emergence from the pupa case) than from their control sisters,[1] the ratio of males to females being about the same as in the case of normal non-disjunction. Nothing is known about the effect of external factors on the production of binucleated eggs, and in any one experiment the conditions may at some time be favorable to one mode of origin and not to the other.

The experiment with marked stock was carried on partly in New York and partly in California, under different conditions, for example of temperature and of moisture. The erratic appearance of composites at certain times will be noted in connection with the account of the mosaic females.

The number of gynandromorphs by single elimination during the whole experiment was less than average and composites from binucleated eggs were wanting.

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[1] Mavor's (1924*b*) evidence for the production of gynandromorphs by the influence of X-rays is not conclusive.

The discovery of composites that developed probably from binucleated eggs in *Drosophila* has come from other sources, but at the same time there have also been new discoveries of elimination.

5. SINGLE ELIMINATION AND COMPOSITES

The kind of elimination that has most frequently been found to produce composites is that resulting in gynandromorphs by the loss of an X-chromosome from part of an embryo developing from a 2N zygote; recently other kinds of composites have been found that are almost certainly due to single eliminations.

VIABILITY

The data indicate that the limit to the kinds of composites derivable by elimination which is imposed by viability of the composites, depends upon the viability of each of its component parts separately.

EFFECT OF LETHAL GENES

Among gynandromorphs by single elimination in lethal stocks there are recorded, besides those in notch stocks, two or three which were formed from zygotes heterozygous for a lethal gene, but only one has been found in which the lethal gene was present in the male region. The gynandromorph occurred in a stock in which males having the lethal gene were perhaps not far below the point of viability, for they sometimes reached the full-grown larval stage. (The gynandromorph in question, No. 4241, was not formed by simple elimination and will be referred to again later.)

EFFECT OF NUMBER OF CHROMOSOMES

Lethal effects are known to be produced not only by specific genes but by various numerical combinations of chromosomes. Elimination from various combinations of chromosomes, none of them carrying a lethal gene, has produced composites, and in all of those so far recorded the resulting numbers of chromosomes in both of the component regions are (with a single exception) known to be viable when present in an entire individual.

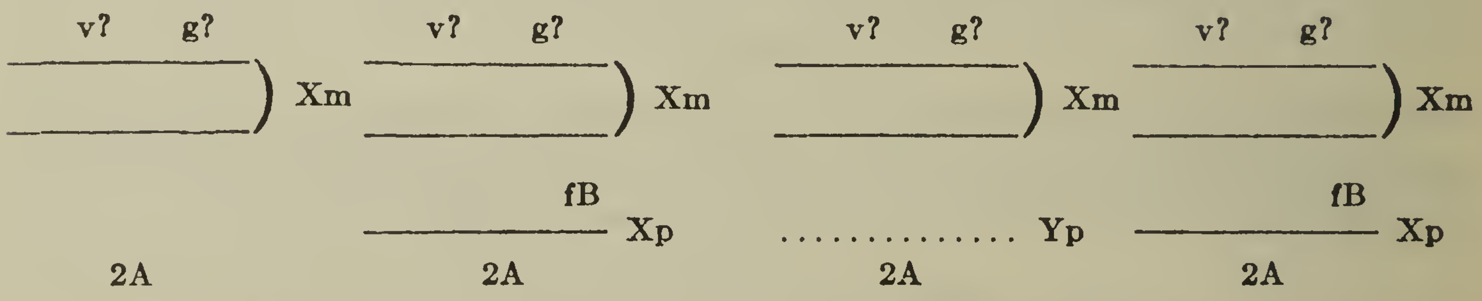
COMPOSITES PARTLY XO, XXY OR HAPLO-IV

The ordinary gynandromorph is the most common example of such composites. It is composed of an XX female region and an XO male region, which in number of chromosomes is like the males that are produced by non-disjunction. Some gynandromorphs, found especially in high-non-disjunctional stocks, if they have been derived by single elimination from regular exceptional XXY zygotes have a Y-chromosome in the female region which is then like an XXY non-disjunctional female (they will be referred to later as non-disjunctional gynandromorphs). In some non-disjunctional gynandromorphs, there has been genetic proof that a Y-chromosome was present in the male region with the X. All of these are viable combinations.

Another example is the class of composites described by Bridges (*see* Gen. of Dros., 1925) in which a 2N region is associated with a haplo-IV region as the result of the elimination of a IV-chromosome. Entire haplo-IV individuals had already been found and described (Bridges 1921).

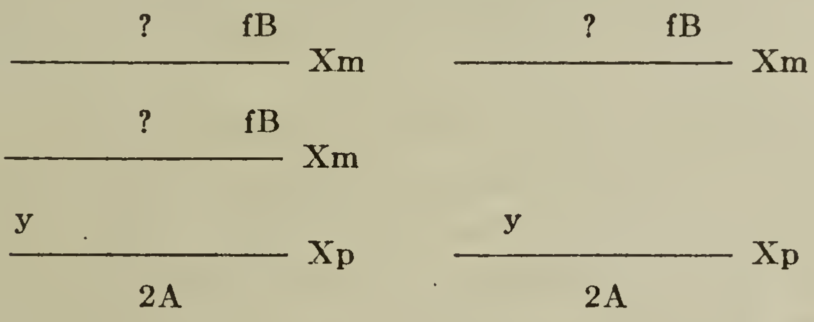
COMPOSITES OF 3X2A-2X2A

Triplo-X females, or super-females, which are 2N in constitution except for the addition of an X-chromosome, are poorly viable, but nevertheless sometimes survive. An example of probable elimination of an X from a triplo-X zygote, in which two of the X-chromosomes were attached to each other, was a female (No. 8.65*a*) which was mosaic for a not-bar 2N region and a heterozygous bar superfemale region (L. V. Morgan 1925). A binucleated egg, if one nucleus were fertilized by Y-sperm and the other by X-sperm, would have produced the same result.



A mosaic of similar chromosomal constitution (No. 8, L. V. Morgan 1922) developed from a regular egg fertilized by non-disjunctional sperm in which the X's were attached and both carried the gene for yellow. The evidence for this case is very complete and points to one explanation. By elimination of the maternal X carrying the gene for bar (*see* table 7), there resulted a female, mosaic for a yellow 2N region and a heterozygous bar superfemale region as shown by the phenotype and offspring of the XX region and by the phenotype and characteristic irregularity of bristles of the XXX region. The fly was uniform for autosomal characters diagnosed for the XX region by the offspring. She was heterozygous black and dichæte and not-hairless, which involved crossing over in the third chromosome making the chances about 25 to 1 that a binucleated condition of the egg would have been detected. But such evidence from the autosomes was not needed, for if the fly had started from two nuclei, there must have been a chromosome from the mother in both regions. There was, however, no X from the mother in the yellow region, as the mother did not carry the gene for yellow. The remote possibility that the mother was an XXY female and that a second nucleus had received a maternal Y-chromosome and had been fertilized by the sperm which contained attached X's is also disproved; for, the sons of the mosaic from eggs produced by the yellow region were undoubtedly XO males; they had received no sex chromosome (Y or X) from their mother since they were sterile and resembled their father.

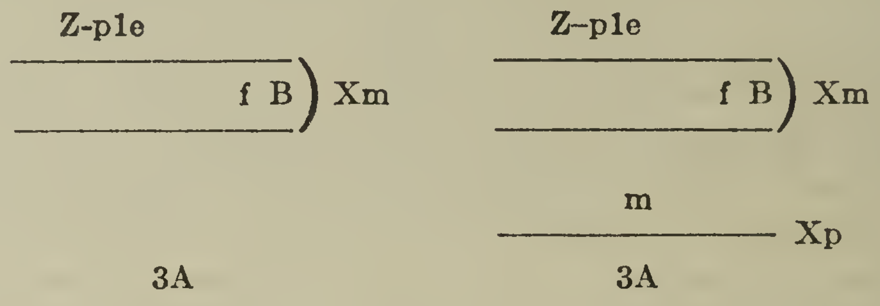
In cultures related to that in which No. 8 was found non-disjunction in egg or sperm occurred several times. The parents of composites that resulted were (like those of No. 8) males which were yellow black and females which had certain mutant autosomal characters and had one sex-chromosome bearing genes for eosin, cut and forked, and one bearing forked and bar. A heterozygous bar female, No. 11 (table 7), one of the offspring of such a mating, was disturbed on the left side in a way suggestive of a triplo-X female. Arista and bristles on the left side were shorter than on the right; the wing was irregular in shape and venation and was held out a little; the left side of the abdomen was irregular. There were no sex combs. Triplo-X females vary in the degree of abnormality, which is in the direction of weakness and smaller size compared with XX sisters; irregularities of bristles are common and also of wings affecting venation, margin and angle of wings. The bar eyes of No. 11 were of unequal width as would usually be the case if one eye were in a XXX region and one in an XX region. Since forked did not show, a paternal X-chromosome (carrying genes for yellow and the normal allelomorph of forked) was present in the broader bar XX region with one maternal forked-bar-bearing chromosome, and in the other XXX region a yellow-bearing paternal X was present with two chromosomes probably carrying genes for forked bar. Elimination of one of the forked bar chromosomes from a zygote derived from a non-disjunctional egg fertilized by X-sperm explains the results. As would be expected, the XXX and XX regions were uniform for descriminating autosomal characters, not-dichæte and not-hairless.



A similar case is probably No. 7 (table 7), which was a female of wild type color with a disturbed left side suggesting a XXX condition. There were no sex combs. On the left side the wing was shorter and so were the bristles on the thorax; on the head there was no difference between the two sides; the eyes were heterozygous bar star and probably both belonged to the XX region. The same numbers of X-chromosomes as in No. 11 would explain the phenotype, but the source of the third chromosome of the XXX region could not be determined; the only character that might have been diagnostic was bar, carried by the mother, which did not affect the XXX region. A non-disjunctional egg or sperm may have supplied the third X. Both kinds of exceptional germ-nuclei had been found to be present three times in related composites. The fly bred as a female, one of whose X-chromosomes carried the genes for eosin, cut, forked and bar (crossover chromosome) and the other the gene for yellow. The fly was uniform for autosomal characters, neither dichæte nor hairless in either region, and heterozygous black in both. Origin of No. 7 or of No. 11 from a binucleated egg is improbable because in both cases one of two egg nuclei must then have been non-disjunctional; the chances were more than 4 to 1 for No. 7 but only 1.6 to 1 for No. 11, that if the two regions were derived from two nuclei it would have been detected. The flies were almost without doubt of the same type as No. 8 and No. 8.65a, that is mosaic of superfemale and female by elimination.

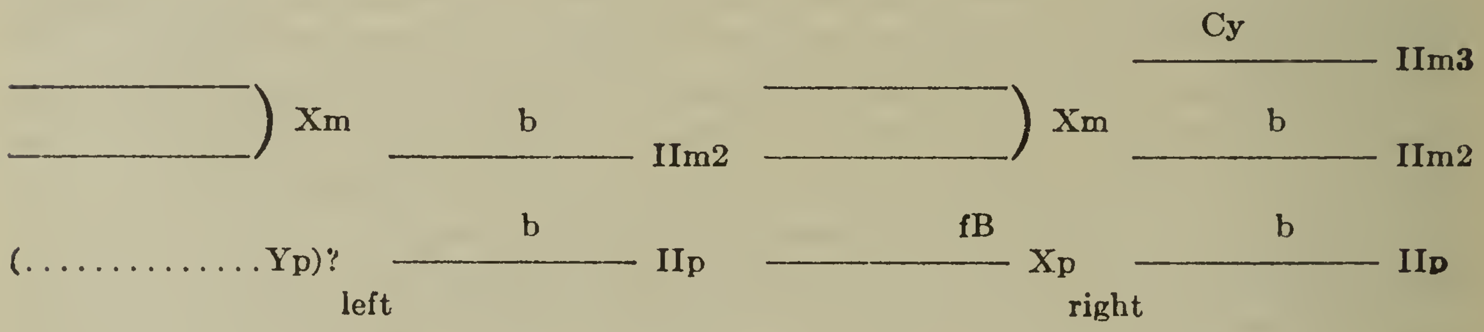
COMPOSITE XXX3A-XX3A

Elimination of an X in a 3X3A zygote probably accounts for a composite (No. 7.10a) previously described (1925). There was formed from a 3N zygote a composite of a bar not-forked 3N female region associated with a forked intersex region that was like one of the regular classes of sibs. As only about 5 per cent of the offspring of attached-X triploid females are attached-X intersexes and only 9 per cent are triploid females, it is not probable that a combination of the two would occur in a binucleated egg.



COMPOSITE 3N-2N

A mosaic female (No. 11.80aa), the offspring of a 3N female, appeared to be undoubtedly derived from two nuclei, because the wings were mosaic for the dominant autosomal character curly. The mother had two attached wild type X-chromosomes and a third X carrying genes for forked and bar; two II-chromosomes carried the gene for black and a third II-chromosome the gene for curly; the father was forked bar, black. The mosaic was 3N on the right side with heterozygous bar eye typical of 3N and curly wing, and was dark in color; the left side was not-bar not-curly and was black, and the fly bred as a 2N black female. Mated to a forked bar black male, the offspring were all black, the males also forked and bar, which is the expectation from a black 2N female with wild type attached X's. If the sons had been tested for fertility, there would have been evidence in regard to the presence of the Y-chromosome.

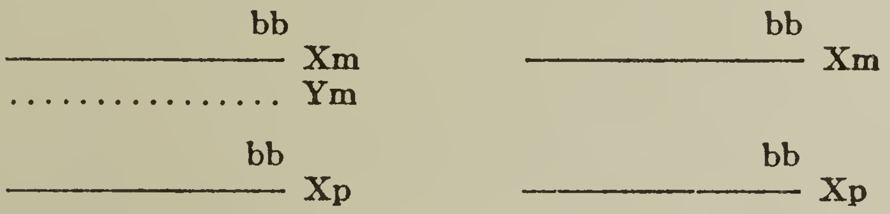


To account for the mosaic and its offspring, the right side of the fly may be supposed to have developed from a diploid egg-nucleus fertilized by X-sperm; one II-chromosome of the egg-nucleus carried curly, the other black, the X's were the two attached wild type maternal X's; the sperm carried the genes for black in the II-chromosome and forked and bar in the X. If the left side developed from a separate egg nucleus, the nucleus had been haploid except for the attached X's, it had received a II-chromosome carrying black, and had been fertilized by Y-sperm carrying black in the II-chromosome. But the possibility that mosaics found by Bridges in the piebald stock resulted from the elimination of a complete set of chromosomes suggests a similar elimination from a regular 3N zygote, of the kind described for the right side. If this occurred, the II-chromosome carrying the gene for curly and the free X carrying genes for forked and bar were two of the set that was eliminated, leaving the left side without curly or bar.

In the case of piebalds, a gene for elimination was present and since there was none in the curly mosaic the explanation by elimination may be less probable.

ELIMINATION OF THE Y-CHROMOSOME

Mosaic females resulting probably from elimination of the Y-chromosome have been described by Stern (1927). He found in bobbed stock, among F_1's from XXY females, three females which were mosaic for bobbed and not-bobbed. The mosaics (Nos. 674, 2674, 951) probably developed from exceptional XY eggs, fertilized by X-sperm, which would ordinarily produce not-bobbed females; by elimination of the maternal Y-chromosome, containing the normal allelomorph of bobbed, a bobbed region resulted. (One of the mosaics, mated to a bobbed male, gave only bobbed females, showing that no Y was present and that the XX region had bred and the test excluded the possibility that the short bristle character was a somatic "minute" mutation.)



INVIABLE COMBINATIONS OF CHROMOSOMES

The inviability of zygotes with an extra one of the two large chromosomes has been shown by failure to develop of diplo-II triplo-III zygotes (or the reverse) from triploid females, nor have biregional flies been found with one such region. Also haplo-II and haplo-III flies have not been found, nor have composites with a haploid II- or III-chromosome region been reported from any experiment. If they existed they should appear as mosaics in crosses in which the father or mother was homozygous for a recessive or both parents homozygous for different recessive genes in the II- or III-chromosomes. Material chosen especially for the purpose of detecting regions haploid for the large chromosomes has been examined with negative results (Morgan and Bridges 1919, p. 10). Mavor (1924*a*) found increased frequency of elimination of the X-chromosome from egg-nuclei after treatment with X-rays, whereas he found no evidence of elimination of a II-chromosome when marked by dumpy in some of the same experiments.

Neither are mosaics with haplo-II or haplo-III regions reported from the piebald stock of Bridges, whereas, besides the numerous gynandromorphs, two mosaics are described which were probably in part haploid for all chromosomes, and explained as due to elimination of a complete set (Bridges 1925*b*). These have already been referred to as the one example of exceptional composites which contain in one region a combination of chromosomes not known to exist independently, but the number of chromosomes being half of that in normal females, the autosomes and sex-chromosomes have a stable relation (*cf.* Bridges 1922). Elimination of an X-, Y- or IV-chromosome, resulting in composites of two viable combinations of chromosomes, occurs with sufficient frequency to give significance to the non-appearance of composites having a region with unequal numbers of II- or III-chromosomes which are inviable combinations. Flies having small mosaic areas due to elimination of an arm of the III-chromosome have been found by Stern[2] (1927*b*).

INDEPENDENCE OF REGIONS IN COMPOSITES

Complete independence of the two regions has always been found in gynandromorphs and it exists also in composites of other chromosomal combinations. This is true for such peculiarities as secondary sexual characters in gynandromorphs (differences in length of wing and bristle, sex combs, etc.), also for somatic effects of a triplo-X condition (for example, irregular venation and other wing disturbances), for somatic effects of haplo-IV (diminished), and for mutant characters, except sometimes vermilion and bar.

The breeding of composites shows that fertility like other peculiarities depends on the constitution of the region bred; 2N may breed when combined with 3N (also a fertile combination), or with XXY; and XY when combined with a different XY region (mosaic males); but a 2N region may also breed when in association with an infertile XXX 2A or an XO region. There are records of mating of 16 gynandromorphs which had apparently normal abdominal XO (infertile) regions, and all failed, and many others have been tried while none bred. There are records of 19 fertile gynandromorphs which had apparently normal female (XX) abdomens out of 34 that were mated. Thus a region, though incomplete as an individual, and associated (as in most or in all of these flies) with infertile XO may be fertile. Breeding may depend not only upon a fertile combination of chromosomes but also upon the inclusion of parts other than the sex apparatus: for example, of the 15 that were mated to males and failed, only two had female heads, 6 had male heads, and 7 had divided heads. Of 19 that bred, 7 had female heads, 11 had divided heads and none had male heads except one which was supposedly male, for it is recorded that the eyes were of the color expected in the male region. No. 8, having a 3X head and thorax bred as a 2X female.

VERMILION

Some new gynandromorphs have been found bearing on Sturtevant's observation (1920) in regard to vermilion. The character vermilion behaved differently from other recessive characters with which it was linked in a fly found by him. It did not show in the genetically vermilion male region of a fly heterozygous for vermilion on the female side. Morgan and Bridges had described three gynandromorphs (Nos. 1373, 4614, 5238 in Group D) with eosin vermilion male eyes, and the question was raised whether eosin was responsible for the difference. In the piebald stock of Bridges (1925), the male spots in the eyes of females were eosin and not vermilion when the genes for both characters were present. The same holds for the eyes included in the male regions of three gynandromorphs (Nos. 18, 22, 17, table 5) found in the experiment just described. Three and perhaps four gynandromorphs (Nos. 3(1), 12b, 15b, 2?), in the high non-disjunctional stock described in section II and perhaps the second gynandromorph of Duncan (1915) were heterozygous for vermilion and had wild type eyes in genetically vermilion male regions. The same is perhaps true for one other gynandromorph of Morgan and Bridges (1919, No. 1333) as will presently be shown. On the other hand No. 113–18, a gynandromorph described in the second section, was heterozygous for vermilion which, with the other recessive characters, is recorded as showing in the male region. No other heterozygous vermilion gynandromorphs with genetically vermilion male regions have been described. No. 1440a (p. 234) was either of that kind and failed to show vermilion, or else the chromosome of its male region was a crossover.

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[2] Cf. Footnote on p. 249.

It appears that as a rule vermilion does not show in the XO region of a gynandromorph, and that the presence of eosin does not necessarily alter the effect. The XO constitution in itself is not accountable for the results, for females heterozygous for the U-shaped chromosome have been mated to scute crossveinless vermilion forked males, and their many non-disjunctional XO sons showed vermilion with the other recessive characters, and also some gynandromorphs homozygous for vermilion had vermilion male eyes. (No. 2134 (Mohr 1923) with an eye in each region, No. 15.8g with two male eyes).

In a mosaic male, No. 937 (Morgan and Bridges 1919), vermilion showed with eosin in one eye while the offspring proved that the normal allelomorph of vermilion had been present in the other region (which also was eosin). A Y-chromosome was presumably present in both regions.

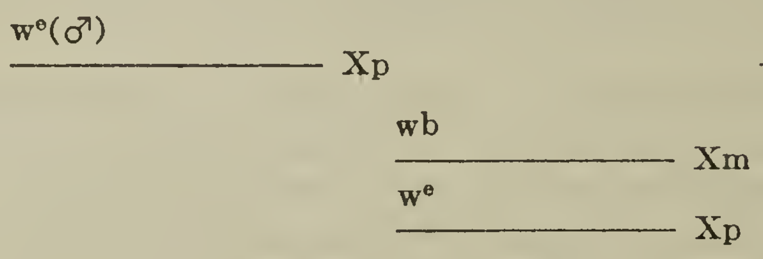
Two gynandromorphs, Nos. 2349 and 192 (*cf.* p. 281), had each a wild type eye in a genetically wild type male region and also a wild type eye in the female region which contained at least one vermilion-bearing chromosome and which was homozygous for another sex-linked character (forked in one and fused in the other fly); these characters had been present with vermilion in one X-chromosome of the respective mothers. The gynandromorphs may be accounted for by assuming that vermilion does not show in a homozygous vermilion female region when the male region contains the wild type allelomorph, or by assuming that the female region had received a crossover chromosome from which vermilion had been crossed out (*cf.* diagrams, p. 281). The abdomens were not entirely female and evidence from offspring from the female region which would be discriminating is lacking.

6. COMPOSITES WITH TWO MATERNAL X-CHROMOSOMES

The composites that have so far been classified are, first, the two unmistakable types, namely, gynandromorphs with Xp in the male region by elimination (type a), and autosomal mosaics from binucleated eggs; and secondly, the ambiguous type b, for which the chances have been found to be in favor of elimination. The composites of more doubtful interpretation are chiefly the "special cases" of Morgan and Bridges for which, as the authors showed, the binuclear hypothesis furnishes a possible explanation. Since the time at which they were described, flies of more than one species of *Drosophila* have been found that probably developed from binucleated eggs. This would make it seem more probable that the flies containing two different maternal X's were of binuclear origin were it not that there is also some evidence for a wider application of the process of elimination, leaving the interpretation ambiguous, as before.

DOUBLE ELIMINATION

A tri-regional composite described by Hyde and Powell (1916) can be explained if, during the development of a female embryo, simultaneous elimination of two chromosomes occurred. The composite was a hybrid



from a cross between a female homozygous for an eye color "blood" and an eosin male. It had a female and a male region, the latter region being mosaic for the two eye colors of the parents. The fly was watched for thirty days and the eye colors carefully compared with those of flies in stocks pure for each color. The binuclear explanation is inapplicable because the fly was tri-regional and in one region the paternal X was without a mate. Another explanation also suggested by Morgan and Bridges fits the case: in an embryo containing one maternal and one paternal X from a regular zygote, two eliminations took place, probably at the same division since the resulting composite was symmetrical: that is, at a certain division the two daughter cells each failed to receive one of two daughter chromosomes.

Admitting the possibility of double elimination, it may perhaps explain certain composites which could not have resulted by simple single elimination including a class which offers special difficulties on the binuclear hypothesis.

If after fertilization of a non-disjunctional egg double elimination should occur at the first division of the zygote triploid for sex-chromosomes, there

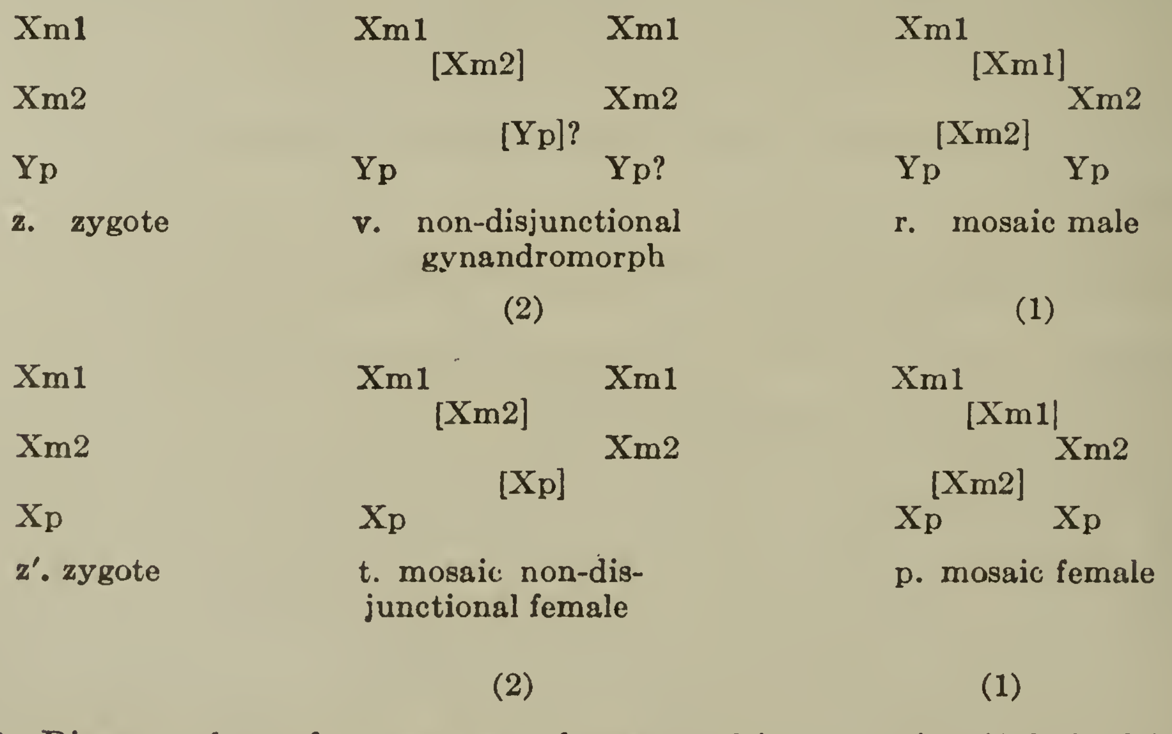


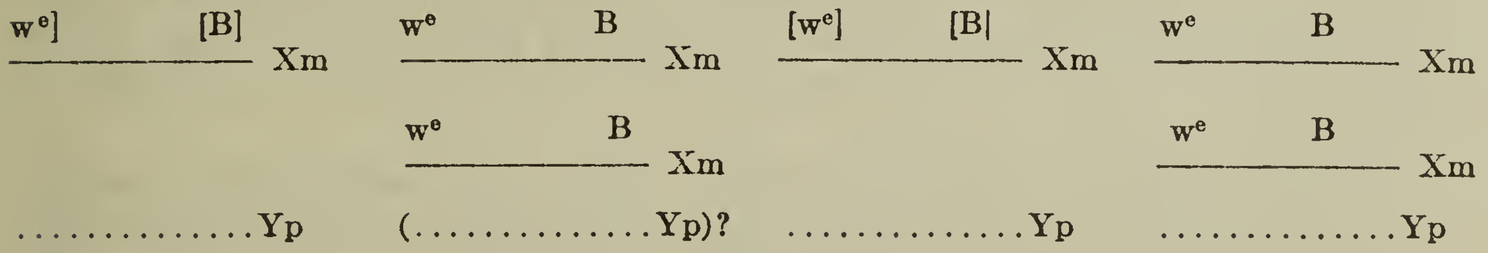
FIG. 3—Diagram of sex chromosomes to be expected in composites if derived by double elimination from a zygote triploid for sex-chromosomes. (The numbers indicate the expectation from fully marked parents; r and p would not be detectable from homozygous females; t. would not be detectable in a backcross when Xm2 is like Xp).

are certain kinds of composites to be expected by chance elimination. These would differ according to whether the paternal chromosome of the zygote was an X or a Y and according to which two chromosomes were eliminated (fig. 3). Even if the chromosomes were marked, elimination of two paternal or of two like maternal daughter chromosomes would not be detected, as no mosaic would be formed and the resulting flies would be either like the regular classes or like the non-disjunctional females, but there are four other classes to be expected (v, r, t and p of the diagram, fig. 3) which if the X-chromosomes were all differently marked would be mosaic. These four classes should, by chance elimination, be two of types v and t to one of types r and p. But from other kinds of matings the expectation is different. If the eggs were of the more usual reductional non-disjunctional type, one half of the class called non-disjunctional mosaic female (type t) would not be detectable in backcrosses that were not marked to show crossing over, nor would mosaic males and females (types r and p) be detectable if the mothers were homozygous. Furthermore, the ratios might be affected by XXX zygotes being less viable than XXY zygotes.

NON-DISJUNCTIONAL GYNANDROMORPH

Composites have been found that can be referred to each of the four expected classes; it is a question however whether any that have been found of type v had been produced by double elimination. The phenotype is represented by a number of gynandromorphs that developed from regular exceptional eggs in high non-disjunctional stocks and a single elimination of an X-chromosome fulfills the requirements of the phenotype.

For example, No. 11a, found in the high-non-disjunctional eosin bar stock (*cf.* p. 232), had a female region like the mother. It was the offspring of an exceptional female and male and is described as an eosin bar female with one sex comb and one shorter (male) wing.



If the head belonged, as is implied, to the female region, the nucleus from which that region developed must have been of the regular exceptional non-disjunctional class, whether the male region developed from the same nucleus (elimination having at some division taken place, fig. 3, type v) or from a second separately fertilized nucleus. If there was but one nucleus fertilized by Y-sperm, a single elimination of an X would account for the result, or Yp might also have been eliminated from the female region without affecting the phenotype; the gynandromorph would then be one of the types to be expected by double elimination.

Similar explanations hold for No. 26a of the same parentage as No. 11a. The gynandromorph had eosin homozygous bar eyes and no sex combs. The wing, last leg and abdomen on the left side were somewhat abnormal. The abdomen and genitalia were mixed, more male on the left and female on the right. Bristles of the mesonotum and of the abdomen were small on the left side. On the head and front legs the hairs and bristles were normal and female on both sides. There were two spermathecæ, a ventral receptacle and at least one very small ovary. Since the eyes were eosin and homozygous bar and the head was female, and since genes for eosin and bar were carried in the maternal chromosomes but not in the paternal, it follows that the gynandromorph developed in part at least from a non-disjunctional nucleus. If the egg had a single nucleus and was fertilized by Y-sperm, a single elimination would have brought about the end result. If the binuclear explanation is applied it is still necessary to assume that one nucleus was non-disjunctional, and both were fertilized by Y-sperm.

Three other gynandromorphs (23a, b and 13b) that were found in the same stock can be explained in the same ways as well as by simple single elimination, as already described (p. 232). The same is true of No. 7673 (table 2, group DI, *cf.* p. 243) also the offspring of an XXY female of a high-non-disjunctional strain.

A gynandromorph from another high-non-disjunctional stock (Bridges 1913, II, p. 600) also admits of the explanations given above. The female region which included both eyes was homozygous for eosin, as was the mother.

[w^e] ———— Xm	w^e ———— Xm	[w^e] ———— Xm	w^e ———— Xm
	w^e ———— Xp		w^e ———— Xm
.... Yp	(............. Yp)?	.... Yp	.... Yp

Almost no flies resulting from double elimination of two X's could have been detected in the stocks in which the XXY females were homozygous for eosin bar or eosin, because mosaic males and females (types r and p, fig. 3) would not have differed from the regular classes, and the last type or non-disjunctional mosaic female (type t) could have been detected only by eye color—that is, in case the head were divided, which reduces the chances of discovery.

No. 7730 (Morgan and Bridges 1919, p. 67), whose mother was a regular female of a high non-disjunctional strain, can also be explained by double or single elimination from a non-disjunctional egg fertilized by Y-sperm if the fly was a gynandromorph. The mother was heterozygous for eosin and forked, and the father was bar-eyed. The interpretation is difficult because only three fourths of the head was of a phenotype that might be female.

w^e(♂) f ———— Xm1	(w^e f ———— Xm1)?	w^e(♂) f ———— Xm1	((w^e f ———— Xm1)?
	———— Xm2		———— Xm2
.... Yp	(........... Yp)?	.... Yp	.... Yp

Again the binuclear explanation can also be used, but only if it is assumed that one nucleus was non-disjunctional. The fly bred as a male but here the test of fertility does not discriminate between the two explanations because, according to both of them, a paternal Y-chromosome is present in

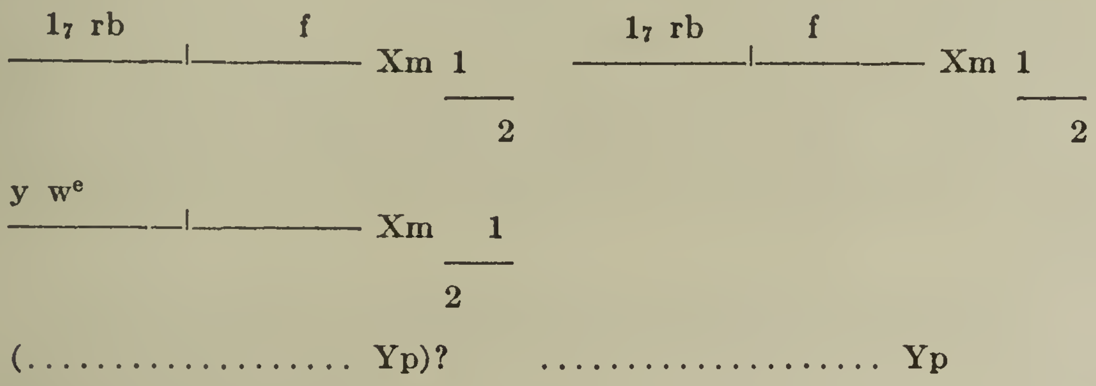
	both	se mos	autosomal mosaic	mosaic male)		
different X's (one or both cross-over chromosomes)	one X-sperm one Y-sperm	sex-linked mosaic gynandro.	n not autosomal mosaic	No. 2349 No. 192 No. 3674 No. 4241 No. 11.13a	n mosaic gynandromorph with 2Xm's	3X's in XXX zygote
			o autosomal mosaic			
	both X-sperm	sex-linked mosaic female	p not autosomal mosaic	No. 5.25a	p mosaic female	Xm1 and Xm2 in XXX zygote
			q autosomal mosaic			
	both Y-sperm	sex-linked mosaic male	r not autosomal mosaic	No. B90 No. 1365 (Mohr '22) No. 1 (Payne '22)	r mosaic male (including 2 of type 1)	2X's in XXY zygote
			s autosomal mosaic			
(one non-disjunctional nucleus must be assumed)				No. G_1a,b2c No. 1 (?) (Dexter '12)	t mosaic non-disjunctional female	Xm1 and Xp in XXX zygote
				No. II (Bridges '13) No. 11a No. 26a No. 7673? No. 13b? No. 23a? No. 23b? No. 4241? No. 7730? (as gyn.) No. 377? (Stern '27*a*)	v non-disjunctional gynandromorph	Xm (and Yp)? in XXY zygote

TABLE 6—*Classification of Composites*

			FROM TWO NUCLEI			BY ELIMINATION		
			chance expectations with complete markings	chances for type with positive diagnosis:			chance expectation	elimination of:
						16 (C II) 9 (D II) 13 (Bb II) (not chance alone) 4 (CII) (not chance alone) 10 (D II) (not chance alone) No. 4 No. 6 No. 14 No. 17 (see type h)	a (2) gynandromorph with Xp in male region	Xm in 2N zygote
X's alike in the two nuclei	one X-sperm one Y-sperm	sex-linked mosaic gynandromorph	b(2) not autosomal mosaic	1:1	autosomes not marked	3 (Ba)	Xp or Xm in male region	Xm or Xp in 2N zygote
				2:1		4 (Ba)		
				3.3:1		1 (Ba)		
				2:1		No. C5		
				6:1		No. 77		
				1:1		No. 113-11 (BbI) (not chance alone)		
				1:1		1 (BbI) (not chance alone)	b (2) gynandromorph with Xm in male region	Xp in 2N zygote
				1:1		1 (BbI)		
				0		2 (BbI)		
				0		13 (CI)		
				0		8 (DI)		
				1:1		5 (DI)		
				2:1		4 (DI)		
				2.7:1		1 (DI)		
				5:1		1 (DI)		
				6.5:1		1 (DI)		
				13:1		No. C3		
				11:1		No. 1		
				30:1		No. 5		
				14:1		No. 15		
				14:1		No. 18		
				2:1		No. 21		
				14:1		No. 22		
			c(30) autosomal mosaic	No. V (G. D.) No. 1734 (Stern) No. 1844 (Stern)	X's not marked			
	both X-sperm	not sex-linked mosaic female	d(1) not autosomal mosaic (not detectable)					
			e (15) autosomal mosaic	No. IV (G. D.) X's not marked				
	both Y-sperm	not sex-linked mosaic male	f (1) not autosomal mosaic (not detectable)					
			g (15) autosomal mosaic					
different original X's in the two nuclei	one X-sperm one Y-sperm	sex-linked mosaic gynandromorph	h (2) not autosomal mosaic	1:1	autosomes not marked	1 (BbII)	a (as above)	
				2±:1		2 (BbII) (lethal in both)		
				2.7:1		1 (BbII)		
				5:1		1 (BbII)		
			i (30) autosomal mosaic	No. 2134 (Mohr '23) crossing-over not detectable				
	both X-sperm	sex-linked mosaic female	j (1) not autosomal mosaic					
			k (15) autosomal mosaic					
	both Y-sperm	sex-linked mosaic male	l (1) not autosomal mosaic			No. 937 (P$_1$ was XXY) No. 7730 (as mosaic male)	l mosaic male	(see type r)
			m (15) autosomal mosaic					
different X's (one or both cross-over chromosomes)	one X-sperm one Y-sperm	sex-linked mosaic gynandro.	n not autosomal mosaic			No. 2349 No. 192 No. 3674 No. 4241 No. 11.13a	n mosaic gynandromorph with 2Xm's	3X's in XXX zygote
			o autosomal mosaic					
	both X-sperm	sex-linked mosaic female	p not autosomal mosaic			No. 5.25a	p mosaic female	Xm1 and Xm2 in XXX zygote
			q autosomal mosaic					
	both Y-sperm	sex-linked mosaic male	r not autosomal mosaic			No. B90 No. 1365 (Mohr '22) No. 1 (Payne '22)	r mosaic male (including 2 of type l)	2X's in XXY zygote
			s autosomal mosaic					
			(one non-disjunctional nucleus must be assumed)			No. G$_1$a,b2c No. 1 (?) (Dexter '12)	t mosaic non-disjunctional female	Xm1 and Xp in XXX zygote
						No. II (Bridges '13) No. 11a No. 26a No. 7673? No. 13b? No. 23a? No. 23b? No. 4241? No. 7730? (as gyn.) No. 377? (Stern '27*a*)	v non-disjunctional gynandromorph	Xm (andYp)? in XXY zygote

the male region. The authors' alternative interpretation of No. 7730 as a mosaic male will be given later.

The same alternative explanations (elimination or binucleated egg) can be used for No. 4241 (Morgan and Bridges 1919, p. 64). The mother carried genes for lethal 7 ruby in one X and for yellow eosin forked in the other. The father was yellow eosin forked. If the gynandromorph resulted from elimination, non-disjunction may have been reductional since the two chromosomes of the nucleus were complementary crossovers. Because the gynandromorph came from a backcross, there were two possible sources for the genes of some of the characters, and on the binuclear explanation only one chromosome was a crossover (diagram, p. 282). For the same reason there is another possible, though not more satisfactory, explanation by multiple elimination in line with that of three or four other gynandromorphs, which will presently be given.



For the non-disjunctional gynandromorphs the presence or absence of a Y-chromosome in the female region would decide whether single or double elimination had occurred, if it could be proved that the egg had had a single nucleus. There has been no test for the presence of a Y-chromosome in the female regions because, in the one only of the flies that was bred (No. 7730), the gonads belonged to a male region. Morgan and Bridges assume the presence of the Y in both regions in their explanation of Nos. 7730 and 4241 by single elimination from non-disjunctional eggs.

For flies homozygous for bobbed there is a genetic test for the presence of a Y-chromosome, because the Y-chromosome carries the normal allelomorph of bobbed.

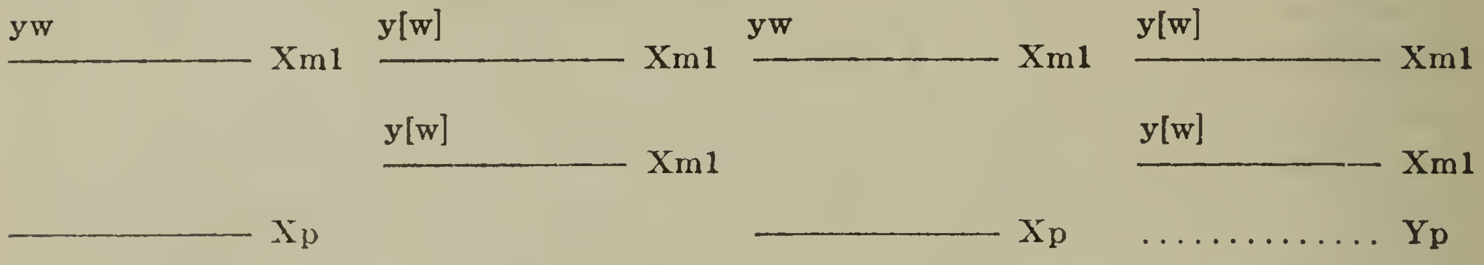
Stern (1927) found several gynandromorphs in bobbed stock that were not bobbed in either male or female region; these he explains by elimination of an X only in an XXY zygote, and their constitution is XXY-XY.

Stern has further described one gynandromorph (No. 377) of a different phenotype which differed also from gynandromorphs (Nos. 47, 76, 780) derived in the usual way by elimination in XX-zygotes; these last were bobbed in both the XX female region and in the XO male region. No. 377 was bobbed in the female region and not-bobbed in the male region, indicating that its constitution was XX-XY. Stern found a high percentage of non-disjunction in the stock and the gynandromorph can be explained as derived from an XXY zygote by the double elimination of an X and a Y. Stern however holds that the regional division of the gynandromorph points to a binuclear origin, in which case also the male region would contain a Y. Only the right side of the abdomen was female. A restricted female region is not however necessarily inconsistent with elimination as shown by an example given in another place (p. 256) of a gynandromorph that was mainly male and explicable only by elimination.

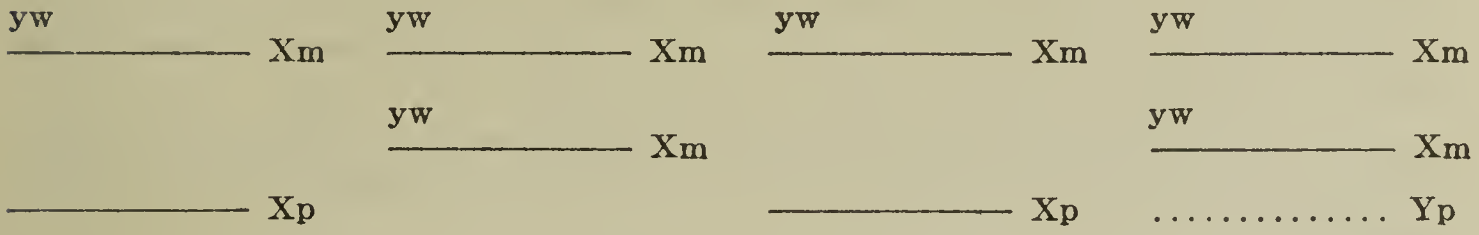
The interpretation by Morgan and Bridges of a gynandromorph of Abraxas reported by Doncaster (1916) may be recalled in connection with single elimination of X in XXY zygotes. The gynandromorph was the offspring of a grossulariata female (ZW) and a lacticolor male (ZZ). It was altogether lacticolor, although lacticolor is recessive to grossulariata. Morgan and Bridges (p. 86) offer the explanation that the zygote contained two lacticolor Z's from a non-disjunctional sperm, and by elimination of one of them there resulted the female region, also lacticolor. Non-disjunctional sperm was known to occur in Abraxas. The interpretation is parallel to the alternative of single elimination offered for the above gynandromorphs of *Drosophila.*

MOSAIC NON-DISJUNCTIONAL FEMALES

A second class of composite having a non-disjunctional region is the mosaic non-disjunctional female, of which an example is No. G_1a, b_2c (Morgan and Bridges 1919, p. 69, *cf.* fig. 3 and table 6, type t). The mother was a white-eosin compound female carrying the genes for yellow and white in one X and for eosin in the other. The father was wild type for sexlinked characters. The gene for the normal allelomorph of yellow carried by the paternal X was present in the mosaic as shown by a not-yellow female region that produced wild type and yellow white sons and few of crossover classes. But a yellow region, also female, showed that two maternal X's were present. A single non-disjunctional nucleus fertilized by X-sperm would have produced a zygote with all the chromosomes whose presence was shown by the composite, but from such a zygote the mosaic could not have resulted by single elimination: the assumption of the elimination of two chromosomes is required (fig. 3, t). If this elimination occurred later than the first divisions of the egg, the fly might have been tri-regional (the phenotype of the triplo-X region not, however, different in mutant characters from that of the wild type XX region after elimination, which bred as an XX female). If the fly developed from an egg with two nuclei, one nucleus must have been non-disjunctional; the improbability of the coincidence of two such rare events as two nuclei in one egg and primary non-disjunction which would be necessary on the binuclear hypothesis fairly excludes that explanation.



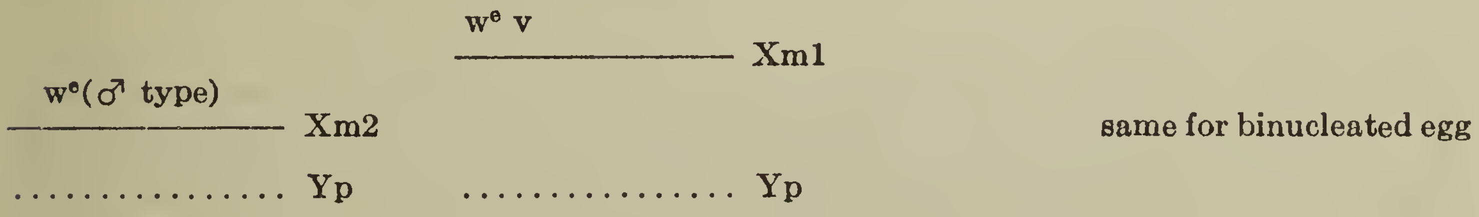
A mosaic described by Dexter (1912) as a female with a white eye on the yellow right side and a red eye on the wild type left side can be similarly explained although Morgan and Bridges have interpreted it as having been male in the yellow white region, and have explained it by simple single elimination resulting in a gynandromorph. The mother was heterozygous for yellow white, the father was wild type.



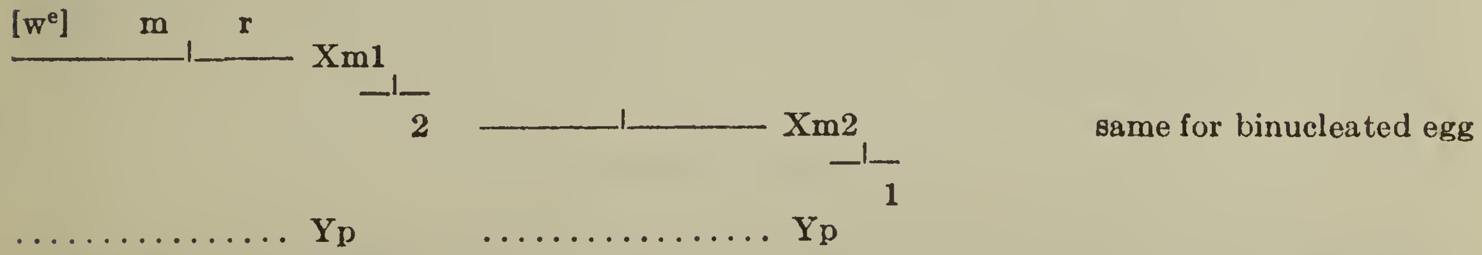
MOSAIC MALES

The other two classes to be expected by double elimination are the unisexual mosaics having two maternal X-chromosomes, one in each region; hence having no non-disjunctional region. Such mosaics have been found, in which the chromosomes were the two different X's of the mother or one of the original X's and a crossover. If they were binuclear in origin, they can be simply explained as the types which have received different X's in the two nuclei, fertilized by two Y-sperm (mosaic male) or by two X-sperm (mosaic female) (table 6, types p, l and r). If they were derived from a single nucleus, the egg was non-disjunctional, the zygote was triploid for sex chromosomes and double elimination was necessary (fig. 3, r and p).

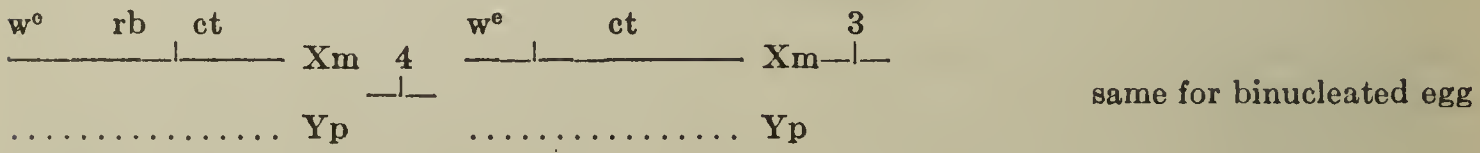
The mosaic male, No. 937 (Morgan and Bridges 1919, p. 61, *see* table 6, l) was a fly whose mother and grandmother were XXY females. The mother was homozygous for eosin and carried also the gene for vermilion in one X-chromosome. The father was vermilion. The mosaic can be explained if an exceptional egg fertilized by Y-sperm underwent double elimination, the Y-chromosome remaining in both regions of the mosaic. Proof of a Y-chromosome in the eosin region was obtained, for the fly mated to a vermilion female was fertile and gave in F_1 and F_2 the classes expected if it were eosin male. The authors give as an alternative the binuclear explanation which presents no difficulties and requires the same chromosomal constitution.



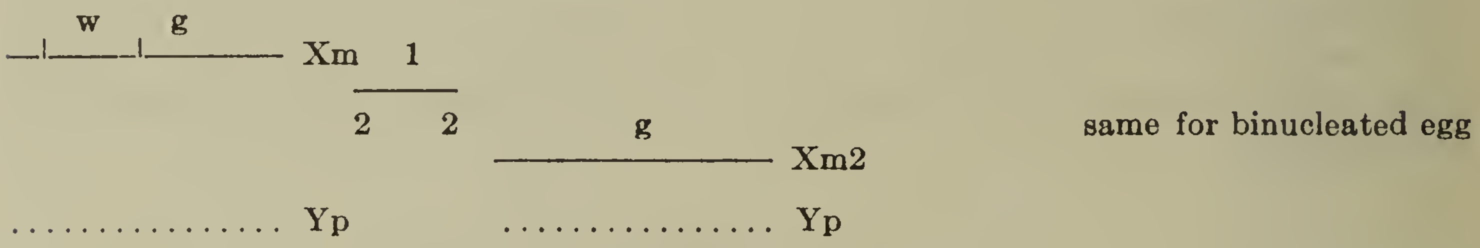
No. B90 (Morgan and Bridges 1919, p. 58, *see* table 6, r) admits of the same explanations if the Xm's that passed into one or into two nuclei were complementary crossovers. Genes for eosin miniature were present in one X-chromosome of the mother and for rudimentary in the other. The father was rudimentary.



Mohr (1922) described a mosaic male (No. 1365, table 6, r) which he suggested may be most simply explained by somatic reversion of ruby to not ruby in a chromosome of one region. The mutant genes carried by the mother were eosin ruby^2 singed in one X-chromosome, and scute echinus cut^6 in the other. The father was eosin singed. If the mosaic was derived from two nuclei both X's as he has shown were crossovers, one of them between ruby and cut, the other between echinus and ruby. If the two crossover chromosomes entered a single non-disjunctional nucleus, the explanation by double elimination given for the last two mosaics can also be applied.



Payne and Denny (1922) have described a composite from a yellow white male mated to a female carrying genes for yellow and white in one chromosome and for garnet in the other. The composite was a fertile mosaic male (table 6, r) with one white garnet eye (the same shade as white) and one garnet eye. Garnet was proved to have been present in the chromosome with white by the offspring which, from a mating with a garnet female, were all garnet, while in F$_2$, white (garnet) males appeared in equal numbers with garnet males. The mosaic is explained if double elimination occurred from a triploid zygote which contained a double crossover chromosome bearing the genes for white and garnet and a garnet-bearing chromosome from the mother and a Y-chromosome from the father. The binuclear explanation is also in accord with the data if the maternal chromosome in one region was a double crossover. The authors have suggested reversion of white to normal or inactivation of the white gene in the garnet region.



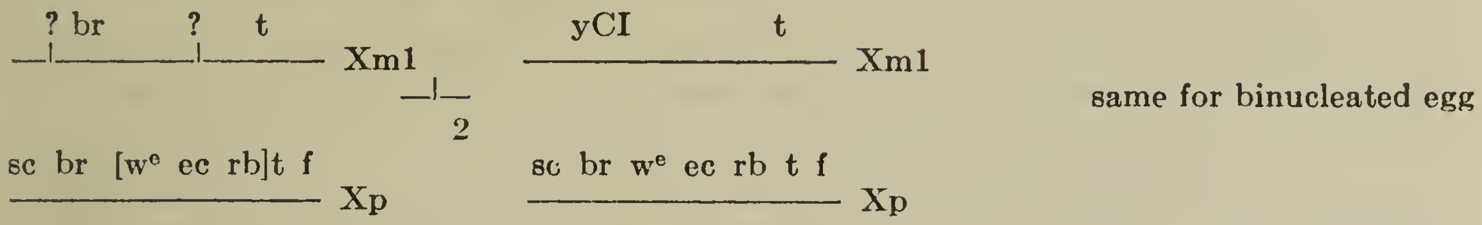
No. 7730 of Morgan and Bridges (*cf.* table 6, l), which has been referred to among non-disjunctional gynandromorphs (p. 272), was thought by the authors to be possibly a mosaic male; if so, the explanations just given for mosaic males are applicable if neither of the Xm's was a crossover. The fly occurred in a high non-disjunctional strain, but there were no exceptions unless the gynandromorph had developed from a single nucleus and therefore was one. The regional division was so extremely uneven that both double elimination and the binuclear explanation seem improbable.

Four of the five mosaic males were mated and were fertile as would be expected by either explanation, since by either origin both regions would contain a Y-chromosome.

MOSAIC FEMALE

Double elimination from a non-disjunctional egg nucleus fertilized by X-sperm instead of Y-sperm would result in a mosaic female (type p). A fly, No. 5.25a, which seemed to answer this description, was found in a

U-strain consisting of females carrying the gene for yellow in a curved chromosome, backcrossed to "7-ple." It showed the presence of two different chromosomes from the mother, and showed also that a paternal X-chromosome was present in both regions.



The diagnosis of the left side offers some difficulties. The fly was apparently female and tan throughout and not-scute, -broad, -eosin, -echinus, -ruby or -forked except that the left wing was broad; one bristle was lacking on the scutellum but not the last as usual in scute, a bristle on the left thorax was somewhat disturbed, but not typically forked (the three characters affecting the eyes were not diagnostic on the left). The maternal chromosome of the left side was either the 7-ple chromosome or a crossover carrying broad or echinus which also gives a broad effect to the wing. If the wing belonged to a male region (by elimination of the maternal chromosome) the neighboring parts would be expected to show forked. Although the diagnosis for 7-ple characters other than broad was not entirely satisfactory, any doubt about them affected only the question of whether, or at what level, crossing over had occurred, if the fly as it appeared to be was wholly female, because in any case a broad and a not-broad female wing indicated the presence of two maternal X-chromosomes and the paternal X. The offspring of the fly, mated to a forked bar male, were 33 heterozygous bar and 40 forked heterozygous bar females, and 35 yellow (tan?) and 35 7-ple males, 1 sc br w^e ec rb t and 1 yf male, and in addition 5 forked bar males. Four of the forked bar males were mated and failed to breed indicating that, conformable to expectation, they were XO males by non-disjunction. The count is typical for F_1's from females having the curved chromosome carrying genes for yellow and tan. One region of the mosaic was therefore shown by the progeny as well as by the phenotype to be XX, and the other side was to all appearances also XX, with full-sized thorax and bristles and no sex comb, and was homozygous for broad (or echinus) but not for other recessive characters of the parents, if there was no mistake in the diagnosis. If the two Xm's had entered the egg in one non-disjunctional nucleus, double elimination was required to produce the two different XX regions. The binuclear explanation, if one nucleus received a crossover chromosome of the kind described fits as well.

Flies of the last two classes, or unisexual mosaics (except 7730), find a more simple explanation on the binuclear hypothesis than by double elimination. But if double elimination may occur, as would appear from the tri-regional composite found by Hyde and Powell, it furnishes an explanation for the non-disjunctional mosaic female No. G_1a, b_2c (and Dexter I?) of type t (fig. 3) where the binuclear hypothesis fails because of the necessity of assuming non-disjunction to explain one of the two nuclei of a binucleated egg. If double elimination is the true explanation in this case, then the other three classes (mosaic males and females and non-disjunctional gynandromorphs) are also to be expected by double elimination. The numbers of flies of the four classes are too small to supply data for a definite conclusion, but as far as they go they are fairly in accord with the expectation by double elimination. Assuming that composites are sometimes produced by double elimination, difference in viability of XXY and XXX zygotes may be a factor in determining the frequency of different types so produced. The numbers of recorded composites possibly derived by double elimination from XXX zygotes are one or two non-disjunctional mosaic females (type t) and one mosaic female (type p) equality being expected (*cf.* fig. 3). Comparison of the numbers of composites from the other kind of zygote (XXY) introduces two difficulties; double elimination of X and Y is not usually distinguishable from single elimination of X, and it is not known whether the chances are even for the elimination of X and Y. If elimination is the explanation of Stern's gynandromorph that was bobbed female and not bobbed male, it is an example of double elimination of an X and a Y in XXY. Otherwise there have been recorded from 3 to 9 non-disjunctional gynandromorphs (single or double elimination, type v), and 4 or 5 mosaic males (type r). Making a comparison in a different way, it appears that two types not involving elimination of a Y, that is mosaic males (type r) and mosaic females (type p), are expected with equal frequency; the recorded examples are 4 or 5 to 1, the type derived from the zygote of greater viability being in excess.

Of the four types only these last two are to be expected if it is assumed that the composites originated from binucleated eggs, because it is highly improbable that the other two types with non-disjunctional regions should arise in that way. The frequency expectation when two nuclei are concerned depends upon chance fertilization by X-sperm and Y-sperm of two nuclei containing each a maternal X-chromosome, and the expected ratio is accordingly one mosaic male to one mosaic female; to these two mosaics are also expected two composites of a third type derived from two nuclei, one fertilized by X-sperm and the other by Y-sperm, resulting in a gynandromorph. Five gynandromorphs of such a phenotype (described in section 7) have been recorded and the observed ratio of the three types is 5 males to 1 female to 5 or 4 gynandromorphs (one having some ambiguous characters). It is to be noted that whereas recombination of characters occurs only in about 50 per cent of X-chromosomes, three of the mosaic males and possibly the mosaic female contained at least one crossover chromosome and the gynandromorphs probably all contained one original and one crossover maternal X-chromosome which is characteristic of equational non-disjunction. The only mosaic male (except the doubtful case, No. 7730) in which the two original maternal chromosomes were present was the offspring of an XXY female and may have developed from a regular exceptional egg.

Besides the deviations from expectation based on sex-linked characters it appears that autosomal mosaics found in miscellaneous experiments are too few in comparison with sex-linked mosaics to attribute all of the latter to binucleated eggs.

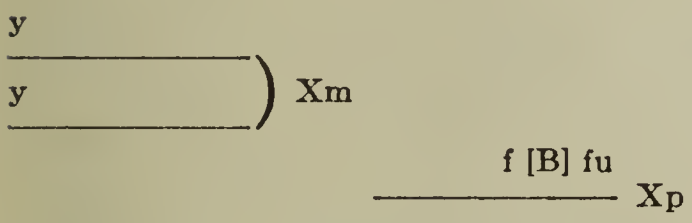
Another explanation besides the binuclear hypothesis is then needed for some of the sex-linked mosaic composites if there is any significance in discrepancies on the binuclear hypothesis in three directions, which may be summarized as follows: the expected ratio of mosaic males to mosaic females to sex-linked mosaic gynandromorphs containing two different maternal-X's and a paternal-X based on chance fertilization by X-sperm and Y-sperm is 1:1:2, the observed numbers are 5:1:5. Autosomal mosaics are too few in comparison with these classes. The expectation for sex-linked mosaic composites containing the original maternal X's, based on chance occurrence of crossover and non-crossover chromosomes, is not realized.

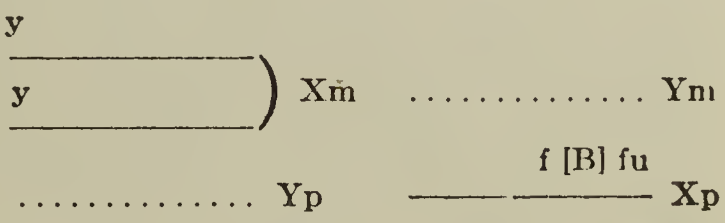
The explanation of the unisexual mosaics by double elimination has been considered; the possibility that the gynandromorphs containing two different Xm's and Xp resulted from triple elimination will be considered in section 7.

GYNANDROMORPHS WITH ATTACHED X'S

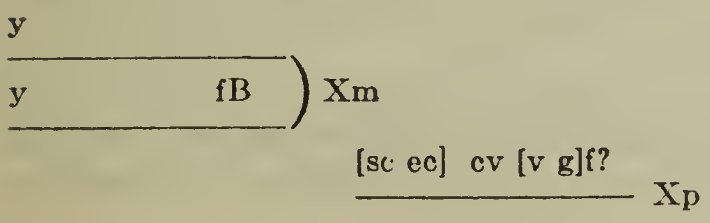
A type of gynandromorph which has occurred when two X-chromosomes were attached to one another can be explained by double elimination or more probably on the binuclear hypothesis.

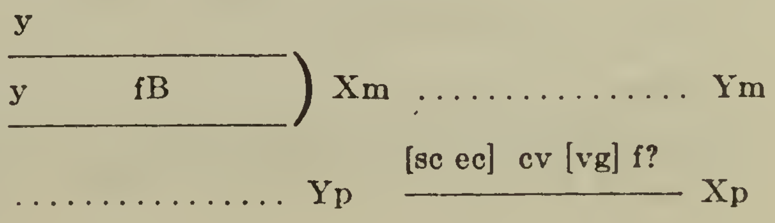
The mother of 3.84c carried the gene for yellow in both of her attached X's, the father was forked bar and fused. The gynandromorph was diagonally divided: the right side of the thorax with a sex comb and the male abdomen were wild type color, the bristles forked and the shorter right wing fused; the head with not bar eyes, and left side of the thorax with no sex comb and longer wing, and a small anterior portion of the abdomen on the left were yellow. The genitalia were twisted; in dissection there seemed to be ovary on one side, and ovary and testis on the other side.





No. 9.30a was the offspring of a yellow heterozygous bar female with attached X-chromosomes, one of which carried the gene for yellow, the other the genes for yellow, forked and bar. The father was z-ple (showing sex-linked characters, scute echinus crossveinless vermilion garnet forked). The head, the anterior legs without sex combs, the thorax, the two posterior left legs, the longer left wing and both balancers were yellow, and so was a very small proximal portion of the right wing. (Sturtevant (1921) has described a gynandromorph of simulans in which the regional line passed through one wing and this occurred also in the gynandromorph No. 1819 described by Mohr 1922.) Both eyes of No. 9.30a were heterozygous bar, not-echinus, -vermilion, -garnet; the yellow region was not-forked, -scute, -crossveinless. On the right the thorax was a little shorter, the last two legs and the crossveinless wing were gray, the wing held a little up and out. The abdomen was male and gray with short bristles (not easily diagnosed for forked).

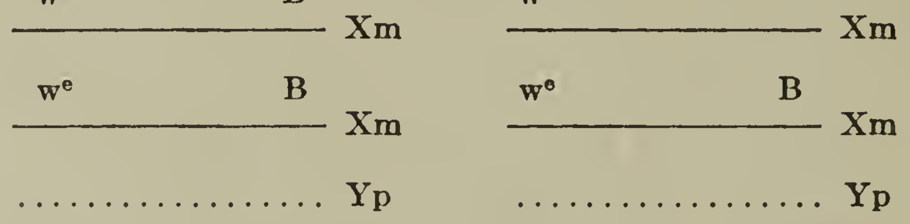




If the last two gynandromorphs developed from a single nucleus, a double elimination must have taken place at the beginning of development because the flies were distinctly not tri-regional: there were two regions, one was female showing the recessive character yellow due to genes of the attached maternal chromosomes, the other region was male (not XXX) showing in one case the recessive characters forked and fused from the father, in the other case the paternal recessive character crossveinless (forked was not easy to identify in the second region which, however, was distinctly male, with shorter bristles and male abdominal markings). The binuclear explanation, on the other hand, is adequate and simple: in attached-X stock there are regularly two kinds of egg nuclei, one containing attached X-chromosomes, the other a Y-chromosome; the binuclear explanation assumes that the two nuclei of the egg were one of each of the regular kinds, and that one was fertilized by Y-sperm, the other by X-sperm. Fertility of No. 9.30, which had a male abdomen, would have proved the presence of the Y-chromosome called for by the binuclear explanation, but on the other hand the failure to breed—which in fact occurred—is at best only negative evidence, and since the head was female, it behaved as have several gynandromorphs in which the head and abdomen were in different sex regions.

UNEXPLAINED MOSAIC FEMALE

A mosaic female (Q), for which there is no satisfactory explanation, may be put on record here. It was the offspring of an eosin bar exceptional female and an exceptional vermilion male of the high-non-disjunctional eosin bar stock (*cf.* p. 229). The mosaic had an eosin bar eye on the left side and an eosin heterozygous bar eye only slightly indented on the right. Mated to a vermilion brother it gave 156 heterozygous bar females, 133 eosin bar males, and of exceptional offspring 10 eosin bar females and 19 vermilion males. The region that bred therefore proved to have contained (like a regular exceptional female) a Y-chromosome and two chromosomes bearing genes for eosin and bar, a region evidently including the left side of the head. A formal explanation dependent, however, on the coincidence of four rare occurrences, is that the fly developed from an egg with two of the regular exceptional non-disjunctional nuclei, and that from one chromosome of one of them bar had been lost by crossing over (reversion); each region

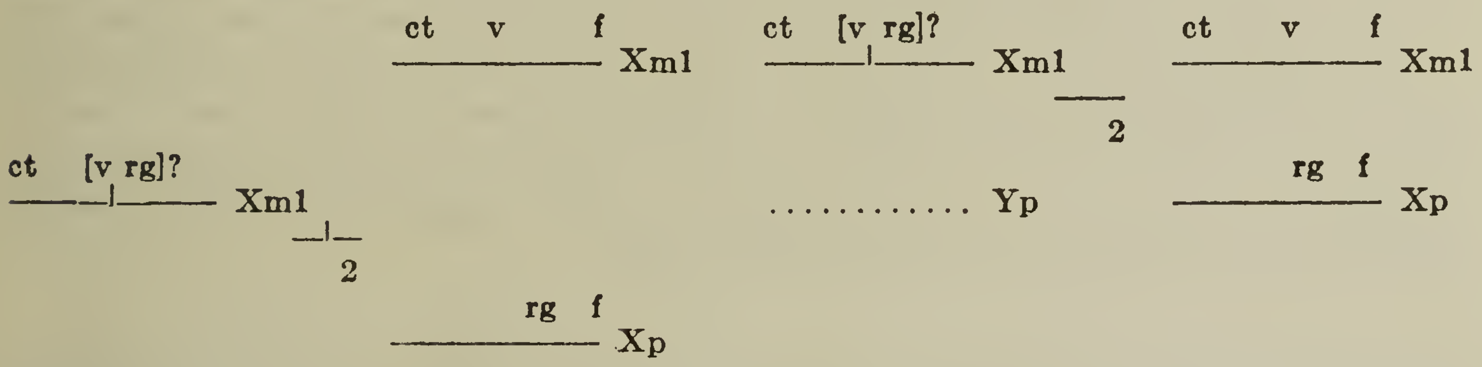


would have contained a Y received from a Y-sperm. The presence of a Y-chromosome in the region that bred was proved by the offspring.

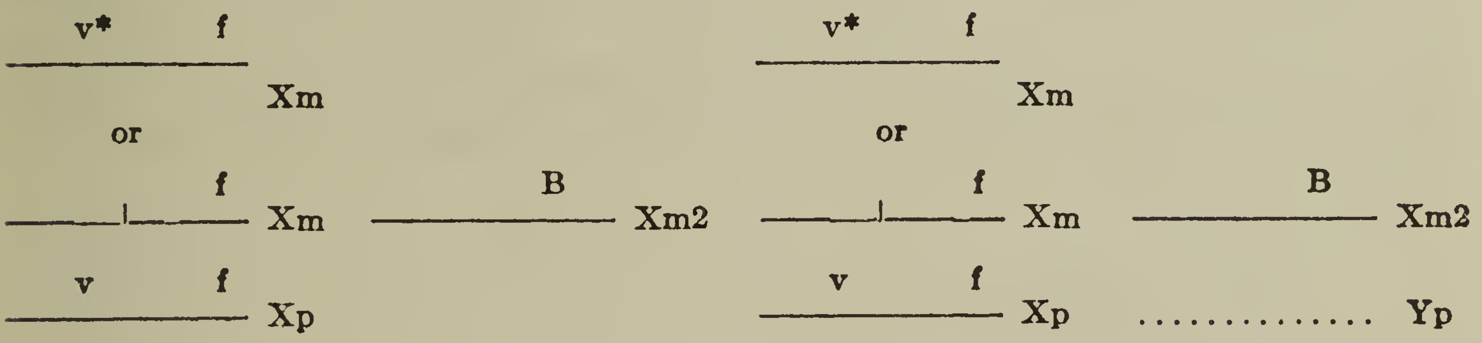
7. GYNANDROMORPHS WITH THREE X-CHROMOSOMES

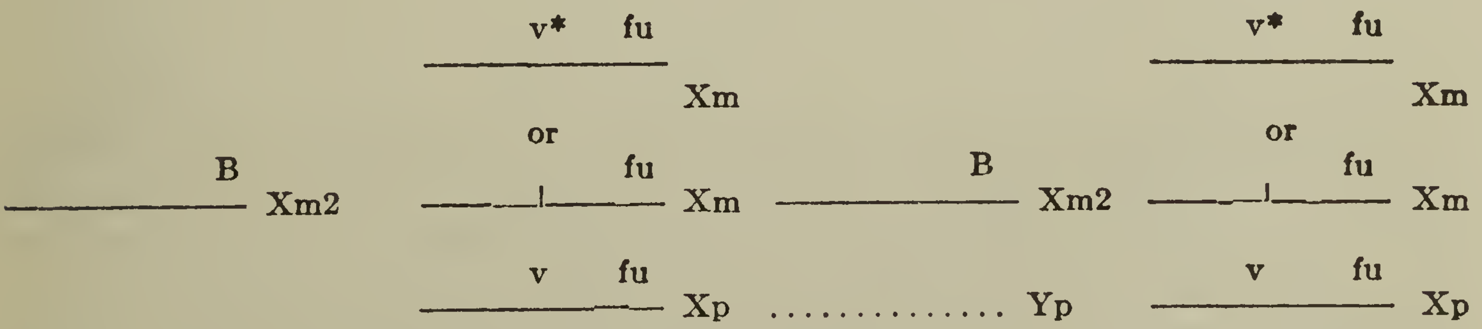
Some reasons have been given for explaining by double elimination the mosaic female and males among the "special cases" of Morgan and Bridges (Nos. G_1a_1 b_2c, 937, B90 and 7730, if entirely male); it will be shown that one of their gynandromorphs, No. 1333, can perhaps be explained by simple single elimination, using Sturtevant's discovery that vermilion does not always show in male regions. Of the special cases there remain to be reviewed three gynandromorphs (No. 3674, and Nos. 2349 and 192) or four gynandromorphs if a new interpretation is given to No. 4241. These and a gynandromorph found recently (No. 11.13a) all showed the presence of three different X-chromosomes; a crossover and a non-crossover maternal X and the paternal X which with one or other of the maternal X's was present in the female region.

The mother of No. 3674 carried genes for rugose in one X and for cut vermilion forked in the other. The father was rugose forked. The distribution of genes in the chromosomes of the gynandromorph is indicated by the following diagram.



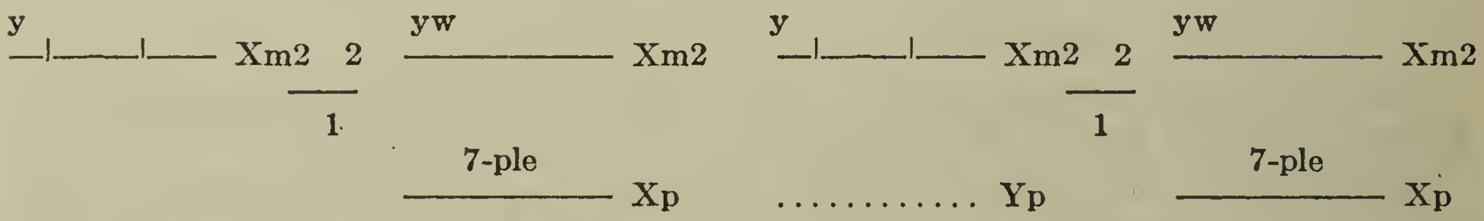
The gynandromorphs include Nos. 2349 and 192, as interpreted by Morgan and Bridges, and already cited in connection with the appearance of vermilion in composites (p. 269). The mother of No. 2349 carried genes for vermilion and forked in one X and the gene for bar in the other. The father was vermilion forked. The parentage of No. 192 was the same except that fused occurred instead of forked. Because the female eye in both gynandromorphs was not vermilion, it is necessary to assume that a crossover chromosome was received from the mother if vermilion would have shown in the homozygous XX region. The male region in both flies was genetically not vermilion.





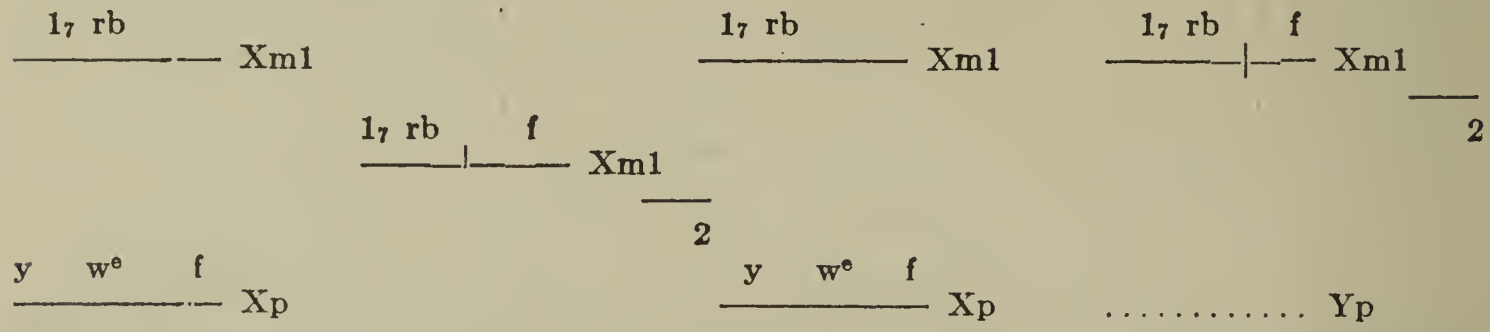
No gynandromorphs are reported with a genetically not vermilion male region, having certainly two genes for vermilion in the female region.

No. 11.13a has not been previously described. One X-chromosome of the mother carried the gene for yellow and also the gene for the normal allelomorph of yellow in a small piece attached at the right end of the same chromosome. The other maternal X carried genes for yellow and white. The mother was not yellow, but wild type color owing to the normal allelomorph carried in the small extra piece. The father was 7-ple. The head of the gynandromorph was wild type with white-eosin compound eyes except for a red spot at the posterior edge of the left eye. The thorax and abdomen were bilaterally divided, yellow and male on the left with sex comb and normal shorter wing, and on the right wild type color with no sex comb and longer wing. The genitalia were yellow and male. The fly was mated and proved to be sterile with females which when mated to other males were fertile. If the red spot in the eye belonged to the yellow male region, that region contained a maternal X that was a crossover because the region was yellow and not white and the right end of the original yellow chromosome had had attached to it the piece carrying the gene for wild type which is dominant to yellow. The light eye on the female side showed that it contained the yellow white chromosome from the mother and the paternal chromosome which carried the gene for eosin.



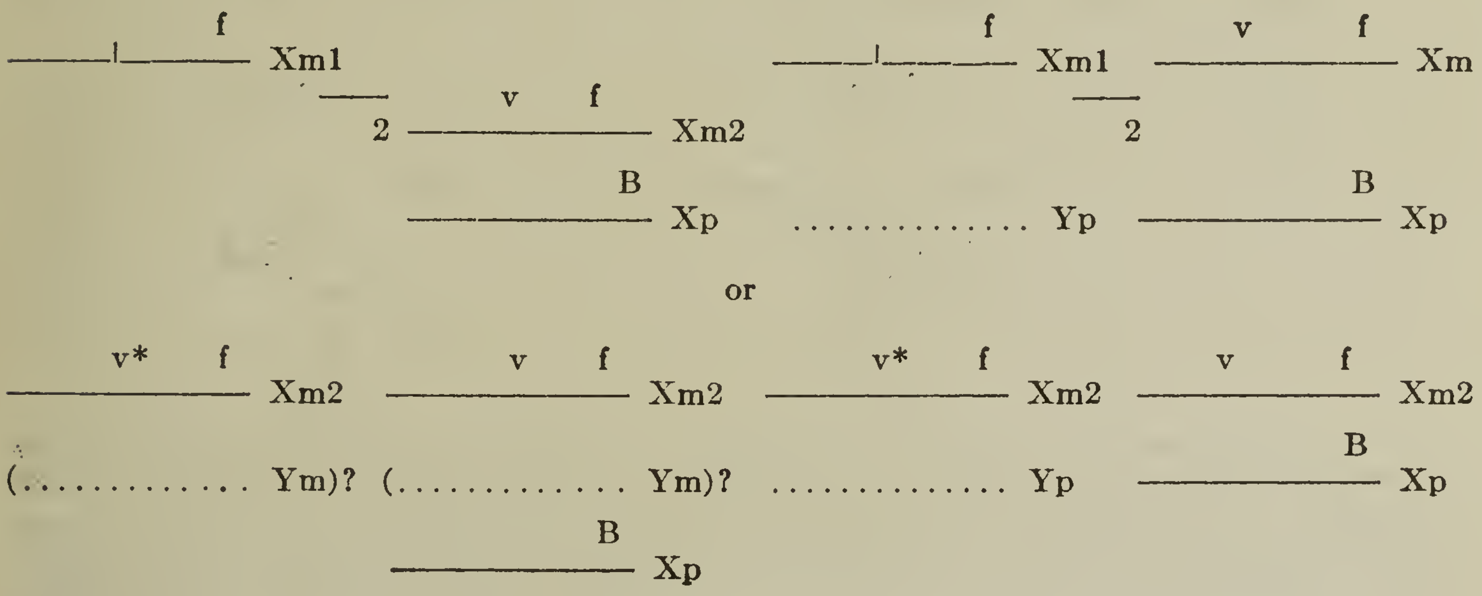
Morgan and Bridges observed that a crossover and a non-crossover chromosome are as a rule found in equational non-disjunctional eggs and that the three gynandromorphs described by them could be explained as derived from such a single nucleus by multiple elimination after fertilization. One of the three (No. 2349) occurred in a high-non-disjunctional stock from a regular mother.

No. 4241 occurred in a backcross, and the explanation is exactly similar to that for the other three gynandromorphs if the mutant characters were, by crossing over, distributed in the chromosomes in the same way as is assumed by Morgan and Bridges for the binuclear interpretation (*cf.* p. 273).



No. 1333 is the last of the "special cases." The mother was an XXY female carrying the genes for vermilion and forked in one chromosome and for eosin in the other. The father was bar-eyed. Morgan and Bridges offer, as one explanation of No. 1333, triple elimination, assuming that a crossover maternal chromosome which carried only wild type genes was the one present in the male region (first diagram); but there is a more simple alternative, in the light of the behavior of vermilion which was not then known.

The presence in the female region of the maternal chromosome carrying vermilion and forked was proved by the offspring, and if it were not expressly stated that there were no forked bristles around the left eye, there would be little doubt that the male eye was genetically vermilion, and that by single elimination of Xp in a regular diploid zygote, the maternal vermilion forked chromosome had been left alone in that region (lower diagram). Offspring of the female region gave no evidence of the presence of a Y-chromosome. On the other hand the regional division of the gynandromorph makes it very improbable that it resulted from two nuclei or by redistribution ot three chromosomes.



That forked had not been crossed out of the maternal X-chromosome of the female region was proved by the offspring. On the whole, the explanation by single elimination seems the most probable.

Leaving out then, No. 1333, it is necessary, if the other four gynandromorphs and 11.13a developed from single zygotes, to assume triple elimination to reduce the number of chromosomes by elimination to the gynandromorph condition of two X's in one region and one X in the other region. Morgan and Bridges pointed out that in none of them did the paternal X remain in the male region as would be expected sometimes by chance elimination. On closer analysis there is found to be no deviation from expectation. The expected frequencies and the different types of triple eliminations are diagramatically shown in figure 4. There are four types of gynandromorphs which might result from an XXX zygote; type b is not distinguishable from gynandromorphs by single elimination of Xm; type c is not different from a gynandromorph by single (or double) elimination from an XXY zygote (*cf*. type v, fig. 3). If all the X's are different, one gynandromorph of type a is expected to two that are diagnostic of type d; in backcrosses the results by triple elimination of type a would be the same as by single elimination, and the same is true for ½ of type d (*i. e.*, when Xm2 is like Xp); from homozygous mothers, type d would appear to have been derived by single elimination. None of the triple eliminations in question were offspring of homozygous mothers; three were from backcrosses, where triple elimination leaving Xp in the male region would appear to be single elimination of the unique Xm; two were offspring of parents with X's all different, when the expectation is one with Xp in the male region to two with Xm. The results then as far as they go do not differ from expectation.

The only gynandromorphs reported of type a (*cf.* fig. 4) are some in which the maternal X's were attached (p. 279), and elimination if it occurred was not strictly triple.

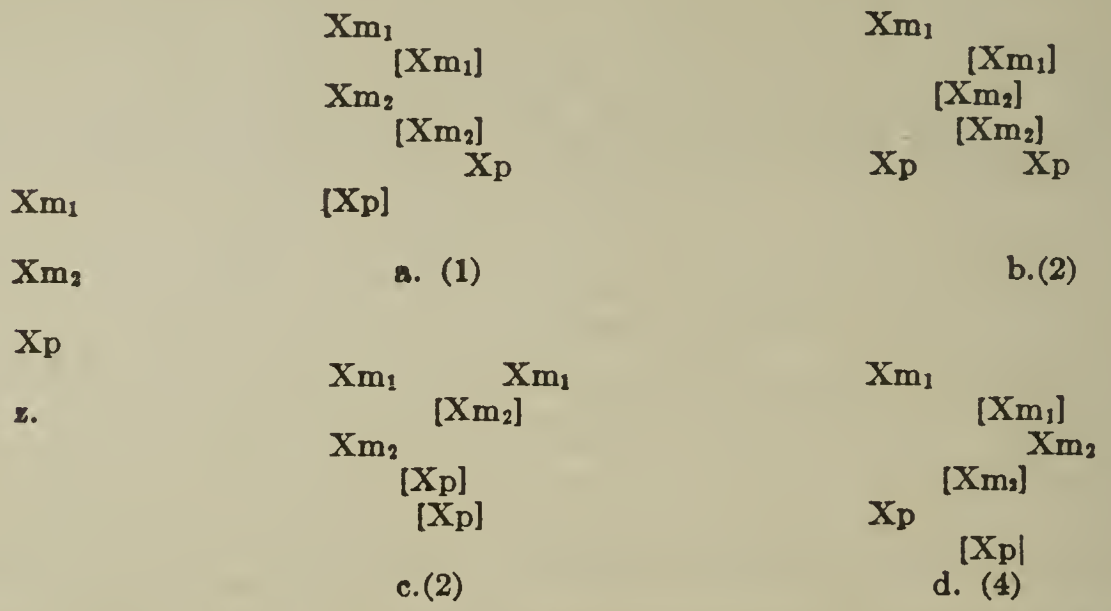


FIG. 4.—Diagram of sex chromosomes to be expected in gynandromorphs if derived by triple elimination from a zygote triploid for X-chromosomes. b. type not distinguishable from single elimination. a. and c. types not distinguishable from single elimination in backcrosses. d. type not distinguishable from single elimination if (with complete markings) the same Xm is eliminated from both cells or if Xp is like Xm_2 (in backcross) or if Xm_1 is like Xm_2 (mother homozygous). When the parental X's are all different, 1 of type a is expected to 2 that are diagnostic of type d.

The phenotypes of the gynandromorphs of type d (fig. 4) are readily explained on the binuclear hypothesis as derived from two nuclei fertilized by X-sperm and Y-sperm; if they had bred as males, there would have been proof of the presence of the Y-chromosome in the male region. There is no record of mating in the account of Morgan and Bridges and probably none of their gynandromorphs had a normal male genital apparatus. No. 11.13a failed to breed as a male, but as the abdomen was in part female, the result of the test is not conclusive. According to expectation on the binuclear hypothesis, there are too many of these gynandromorphs having a crossover X (type n table 6) in comparison with the expected class of gynandromorphs of the type containing two original maternal X's. None of these (type h) have been recorded excluding the possibility that Nos. 2349 and 192 might have failed to show by vermilion color that the maternal X in the female region was one of the original chromosomes, and excluding flies of the stated phenotype that are found in backcrosses and are almost certainly produced by elimination. The absence of type h (if No. 2349 and 192 are excluded) is also at variance with expectation by elimination, in as far as primary non-disjunction is more often of the reductional than of the equational type. If XXX zygotes, resulting when non-disjunctional eggs are fertilized by X-sperm, sometimes survive by elimination, it would seem probable that more gynandromorphs would result from eggs produced by reductional non-disjunction—that is, containing the original X's—than by equational non-disjunction. It is to be noted also that, assuming elimination, these gynandromorphs form a comparatively large class by elimination of three daughter chromosomes in an XXX zygote, and there are very few (if any) composites by elimination of two chromosomes (mosaic female, type p). Four are recorded (p. 264) by elimination of one chromosome in

XXX (resulting in 3X2A-2X2A), but these occur rarely even in attached-X strains in which one regular class of zygote is XXX. Poor viability of one component region may reduce the viability of the last class. The deviation from expectation on the binuclear hypothesis which was pointed out in section 6 seems to be more significant than that shown above for elimination, and multiple elimination appears to be on the whole the more probable explanation for most of the complex gynandromorphs.

The conclusions in regard to types including the "special cases" may be summarized as follows: For composites that are non-disjunctional in one region, elimination (single and in some cases double) seems to be decidedly more probable than origin from two nuclei, and—in spite of the discovery of autosomal mosaics very probably derived from binucleated eggs in *Drosophila,* the evidence is rather in favor of double elimination as one explanation even of unisexual mosaics with no non-disjunctional region. Furthermore it cannot be said that the discovery of composites derived from two nuclei has brought a definite solution for all of the composites of a class requiring a still more complex elimination, that is the gynandromorphs among the "special cases," which contain a crossover and a non-crossover maternal-X and a paternal-X, and require as many as three eliminations. These are probably more numerous than is to be expected on either hypothesis. The expectation for this type as compared with other types from binucleated eggs is based on occurrencies of known frequency, that is chance fertilization by X-sperm and Y-sperm and crossing over. By triple elimination frequencies of reductional and equational non-disjunction are involved and differential viability of zygotes. Binucleated eggs probably do occur and there is no proof of triple elimination, but to these gynandromorphs derivable in either way may be added as possibly due to multiple elimination two composites which developed from embryos containing an even greater number of X-chromosomes, and which are not explicable on the binuclear hypothesis.

8. MOSAIC FEMALES WITH FOUR X-CHROMOSOMES

Four different X-chromosomes were shown to be present, two in each region, in mosaic females for which the binuclear explanation offers no solution, because one of the regions contained no maternal X-chromosome. The two mosaics were nearly related to the mosaics Nos. 8, 7 and 11 (p. 264), all from parents having the same sex-linked characters. The flies that were being examined at the time for composites were from pair cultures of sisters or nearly related females mated to males which, though closely related to each other, were of different stock from the females. It is therefore possible that a temporary frequency of non-disjunction, which affected sometimes eggs and sometimes sperm, was due to some condition of the environment.

No. 9 (fig. 5 and table 7) was entirely female, with parts throughout of equal size including the wings. The fly was to all appearances 2N in both regions. There were no sex combs. The left eye was heterozygous bar and the region around it forked; the left side of the head and thorax including legs and wings was gray and forked, and the abdomen ventrally was gray on the left. The right eye was wild type (not-bar). The right side, includ-

TABLE 7—*Non-disjunctional composite females*

Kind of composite	No.	Parentage	Approximate regional distribution	Constitution	Origin	Fertility
♀—s-♀ mosaic	8	w^e ct f ——Xm_1 ——IIm_1 D H ——$IIIm_1$ fB ——Xm_2 S b ——IIm_2 ——$IIIm_2$ by y ——Xp b ——IIp_1 ——$IIIp_1$ ——Yp b ——IIp_2 ——$IIIp_2$	2X ♀ with 3X head and thorax	fB ——Xm_2 y —— y ——) Xp y —— y ——) Xp $\frac{b}{+}$ $+^S$ D $+^H$ $\frac{b}{+}$ $+^S$ D $+^H$	Non-disj. sperm Elim. of Xm	$\overline{yy}$ $\frac{b}{+}$ D ♀+ 100% non-disj.
	7	as above	2X ♀, probably XXX on left side of thorax	w^e ct fB ——|——$Xm_{\frac{1}{2}}$ w^e ct fB ——|——$Xm_{\frac{1}{2}}$? ——X? y ——Xp y ——Xp $\frac{b}{+}$ [S] $+^D$ $+^H$ $\frac{b}{+}$ S $+^D$ $+^H$	Non-disj. egg or sperm Elim. of an X	w^e ct fB ——|—— y ♀+
	11	w^e ct f ——Xm_1 ——IIp^1 D H ——$IIIp_1$ fB ——Xm_2 ——IIp_2 ——$IIIp_2$ by	bilateral	? ? fB ——Xm ? ? fB ——Xm ? ? fB ——Xm y ——Xp y ——Xp	Non-disj. egg Elim. of an X	♀ —

		y ——Xp b ——IIp_1 ——$IIIp_1$ ——Yp b ——IIp_2 ——$IIIp_2$		$\frac{b}{+} +^D +^H \quad \frac{b}{+} +^D +^H$		
♀ patro-matro-clinous mosaic	9	as above	approximately bilateral except abdomen	w^e? ct? f ——Xm_1 fB ——Xm_2 y ——Xp y ——Xp $\frac{b}{+} +^D +^H \quad \frac{b}{+} +^D +^H$	Non-disj. egg and sperm Elim. of 4X's ?	$y \frac{b}{+}$ ♀ +
	12	w^e ct f ——Xm_1 S b ——IIm_1 D H ——$IIIm_1$ fB ——Xm_2 ——IIm_2 ——$IIIm_2$ by y ——Xp b ——IIp_1 ——$IIIp_1$ ——Yp b ——IIp_2 ——$IIIp_2$	fore and aft division thro' ant. part of abdomen	? f [B] ——Xm? ? f [B] ——Xm? y ——Xp y ——Xp [S] $\frac{b}{+}$ [DH] $+^S \frac{b}{+} +^D +^H$	Non-disj. egg and sperm Elim. of 4X's?	genitalia imperfect

ing head, thorax, wing, legs and the abdomen ventrally and the balancer, were yellow, and the left balancer was also yellow and probably all of the abdomen dorsally. Both wings were not-dichæte and not-hairless.

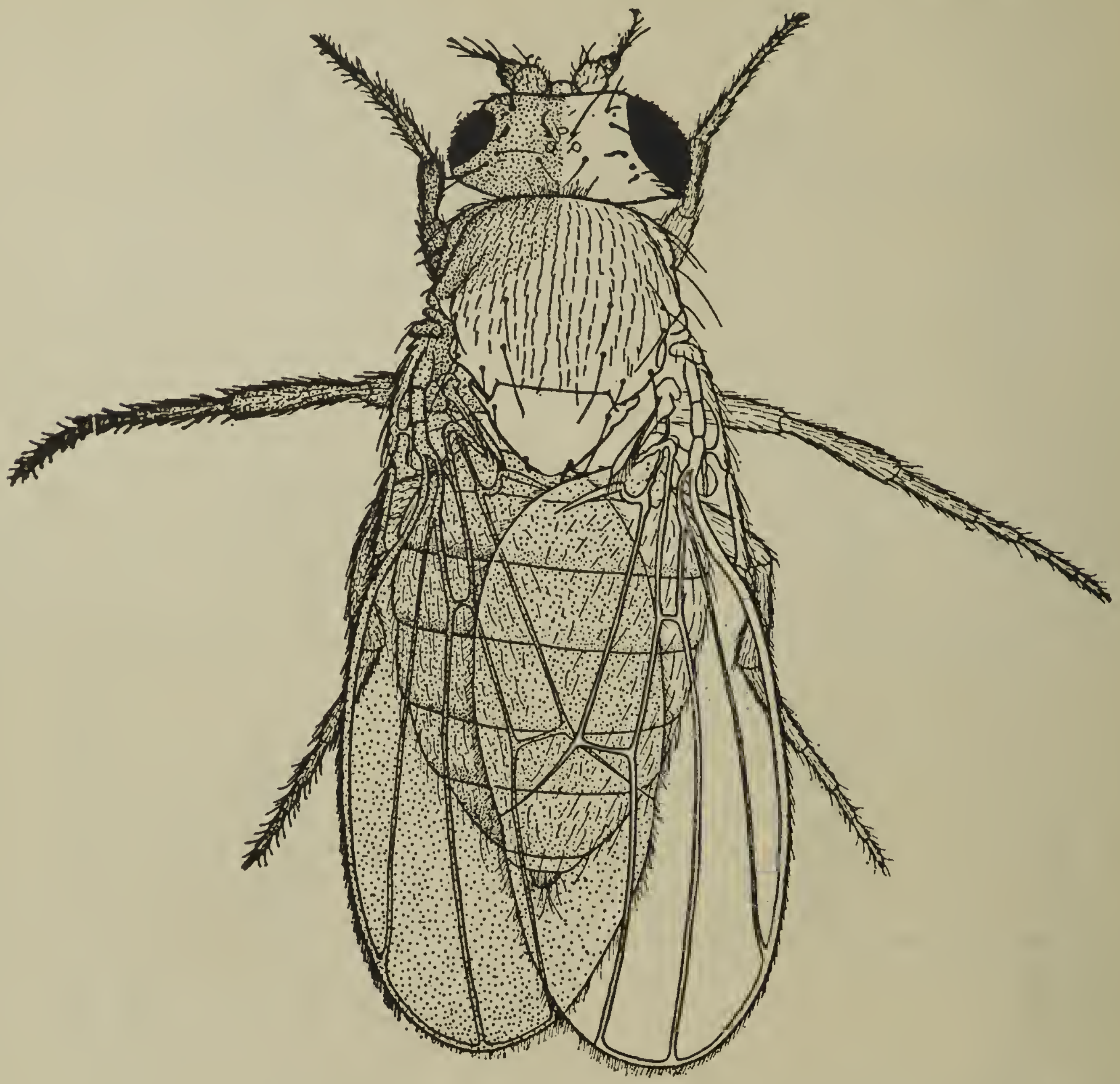
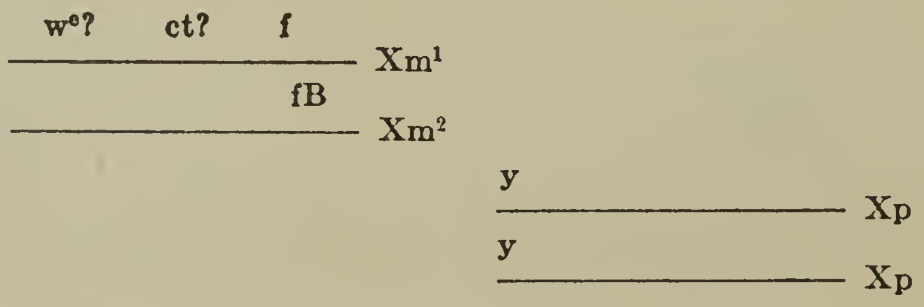
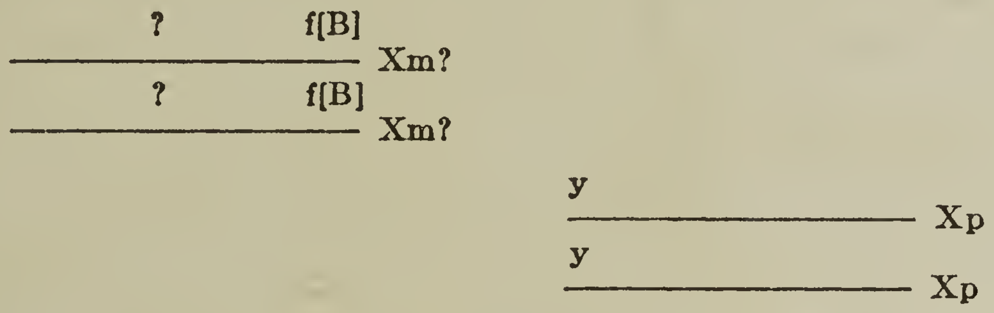


Fig. 5—Patroclinous mosaic female partly matroclinous on the left. The left side of the head and of the anterior part of the thorax, and the anterior part of the abdomen on the ventral side were wild type color and carried forked bristles. The left legs and wing were wild type color. The left eye was heterozygous bar. The fly, except for these parts, was yellow not-forked; the yellow region included therefore the right side, legs and wing, also all of the posterior part of the thorax and both balancers, the dorsal side of the abdomen and all except the anterior left part of the abdomen on the ventral side. The right eye was not-bar.

The ovaries were included in the patroclinous region as shown by the offspring; all of the 23 sons and some of the grandsons were yellow (numbers in F_2 not recorded). The chances were 1.66 to 1 that the autosomes would have revealed a binuclear origin, but the yellow color in a female region showed that no maternal X was present in that region. A single nucleus must have contained four X-chromosomes and if the fly resulted from elimination, two chromosomes were eliminated from each region.



The problem is the same for the next fly, No. 12 (table 7), but there is no evidence from autosomal characters except black; and no evidence from offspring as the fly did not breed. It was female and entirely yellow, except that the last five segments of the abdomen were gray and forked; the genitalia were imperfect. Any two maternal chromosomes, original or crossovers, would account for the gray forked region, since they both carried forked and not yellow, and characters due to the other mutant genes did not affect the abdomen. The yellow color of most of the fly showed that no X from the mother was present in one region which was female and therefore contained two X's from the father.



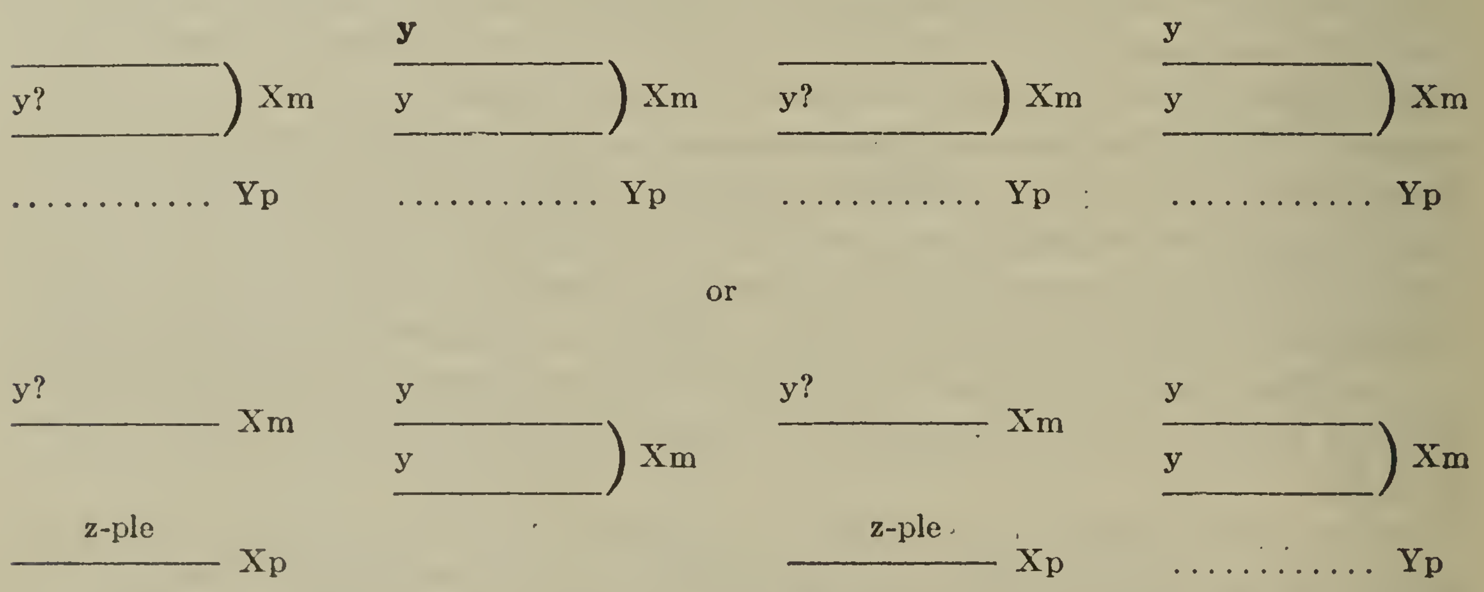
HIGH FREQUENCY OF EXCEPTIONS

The last two mosaics were found during a period of high frequency of elimination which occurred while the experiment with marked autosomes was being carried on in California. On February 9 a simple gynandromorph (No. 4) was found; on February 11 two more (Nos. 5 and 6) were found. The mosaic females, explained above and in section 5 as due to multiple or single elimination following non-disjunction in egg or sperm, were found on the following dates: Nos. 7 and 8 on February 12, No. 11 on February 14, No. 12 on February 21 and No. 9 on March 4. Between February 9 and March 4, inclusive, 2,167 flies had been observed. Previous to this period, December 23 to February 9, two simple gynandromorphs had been found among 3,724 flies. No composites were found among 3,442 from October to December 23, or among 10,143 flies from March 5 to June. Lagging of an X-chromosome may furnish a consistent explanation of some of the steps in the formation of the composites found during the period of frequent exceptions. If, at a stage before or at maturation, a daughter chromosome had been held back to the extent of remaining in the cell with its sister half or (at the reduction division) with its chromosomal mate, the non-disjunctional germ cells that were the basis of some of the composites would have arisen. The elimination of chromosomes in the embryonic stages, resulting from loss of one or more retarded daughter chromosomes lagging behind the other daughter chromosomes of the set to which they belong, would explain the different distributions of chromosomes found in all of the composites including the simple gynandromorphs. Thus a temporary condition favoring lagging may have been a factor in the appearance of various mosaic females and of a high percentage of simple gynandromorphs during a short period.

In so far as single or multiple elimination may be a process compensatory for non-disjunction, the opportunity for it is increased when conditions are favorable for non-disjunction. It does not follow that "lagging" is always brought about in one way. In non-disjunctional sperm in which the two X-chromosomes were attached (No. 8), it followed incomplete separation of two daughter halves of a divided chromosome. Lagging may however be an effect of other factors as for example lagging of a U-shaped chromosome and of chromosomes carrying the genes for minute-n and perhaps for notch or bobbed.

9. MOSAIC FEMALE WITH FOUR X-CHROMOSOMES ATTACHED IN PAIRS

Another mosaic female (No. 751–56) of a different origin from those last described showed that she had received four X-chromosomes attached in pairs from her mother. In this case the origin from two nuclei presents no difficulties because the two nuclei of the single egg would have been like the regular kinds of eggs. The mother was wild type, with two attached X-chromosomes, one wild type and one bearing the gene for yellow. The strain in which the mosaic was found gave 100 per cent patroclinous males showing that the X-chromosomes of the females were attached; the females were of two phenotypes, wild type and yellow; some of the wild type flies of which the mother of the mosaic was one, were heterozygous for yellow for they produced some yellow females by crossing over; the mosaic was composed of two female regions, one wild type and one yellow. She was female throughout with no sex combs; the right side of the thorax except the humeral patch, the right legs and wings, and two anterior segments of the abdomen on the right were yellow. The other parts of the fly (head, left thorax and most of the abdomen) were wild type color. She failed to breed. The right side must have received two yellow-bearing chromosomes from the mother as it was female and the father's X-chromosome carried not-yellow, but the recessive genes called collectively z-ple. The left wild type side may have contained either a wild type combination of attached X's or a broken apart X from the mother and an X from the father. In either case, the zygote if it had come from a single nucleus must have contained four X-chromosomes (in the first case with a paternal Y-chromosome in addition), whereas the binuclear hypothesis gives a simple explanation for either possibility.



The composites that have been reviewed have been divided into two main groups according to their probable origin (*cf.* table 6).

Those probably derived from binucleated eggs are five autosomal mosaics not derivable by elimination and the mosaic female No. 7.51–56 (p. 290) containing two pairs of attached X-chromosomes. Perhaps a very few of the gynandromorphs of the type ambiguous for single elimination were of binuclear origin, and possibly also some of the composites of the types explicable by double or triple elimination.

Composites by elimination are most of the gynandromorphs explicable by single elimination in a 2N zygote (by far the largest class of composites) and composites by single elimination from other combinations of chromosomes (p. 263 ff); perhaps also the mosaic females (p. 285) containing four X-chromosomes (which were certainly not derived from two nuclei), and most of the gynandromorphs derivable by double and triple elimination.

Gynandromorphs Nos. 3.84c and 9.30a (p. 279) with attached maternal X-chromosomes in one region and a paternal X in the other region are not assigned to either explanation; a crucial test for such gynandromorphs, if fertile, is the presence or absence of a Y-chromosome. No. 11.80aa (3N-2N, p. 266) is also not classified.

SUMMARY

1. Morgan and Bridges reported that most of the gynandromorphs of *Drosophila melanogaster* can be explained if it is assumed that a daughter X-chromosome is eliminated from one cell of a regular 2N zygote at an early stage of development. If the eliminated chromosome had come from the egg-nucleus, the male region of the gynandromorph (to be denoted as type a) contained a paternal X (Xp), if it had come from the sperm, the male region contained Xm (type b). The two types were found in about equal numbers.

2. There were nine "special cases," comprising both unisexual mosaics and gynandromorphs (here included under the general term "composite") that could not be explained by simple single elimination because they contained two X-chromosomes that had been received from the mother. The authors suggested two alternative explanations: (1) that multiple, or in a few cases single, elimination took place in a zygote from a non-disjunctional egg, or (2) that the individuals arose from binucleated eggs.

3. An experiment has been carried out which was planned to increase the chances of detecting composites derived from binucleated eggs. Flies were mated whose chromosomes were so marked that the expectation for gynandromorphs by simple single elimination was as usual 50 per cent with Xp in the male region (type a) and 50 per cent with Xm in the male region (type b). The phenotype of the latter class is also derivable from an egg containing two nuclei. But, with the aid of the autosomal markings used in the experiment, less than 2 per cent of possible composites from binucleated eggs would be of the ambiguous type b, and, excluding types that would not be mosaic, genetic evidence for two separate egg-nuclei would be expected in about 97 per cent of all composites of binuclear origin. And of the 97 per cent not explicable by single elimination, if any occurred, less than 4 per cent (table 6, types h, j and l) would be explicable by multiple elimination, as well as on the binuclear hypothesis.

4. There were found, among 74,219 flies, 4 gynandromorphs of type a, 5 gynandromorphs among the counted flies and 1 in a related culture of the ambiguous type b, 3 non-diagnostic gynandromorphs probably derived by elimination (type a or b), and none of the types that are explicable only on the binuclear hypothesis. There were in addition 5 composites involving non-disjunction of egg or sperm, to which the binuclear hypothesis was not applicable.

5. There was evidence that all of the 13 simple gynandromorphs of types a and b had probably been produced by elimination. The 4 of type a were unequivocal because of the presence of the paternal X in the male region, and of type b there was an almost equal number (5 among the counted flies), which is in accord with expectation if they too had resulted from elimination. The 3 not diagnostic gynandromorphs were almost entirely female, which is more in accord with elimination than with the other explanation. Finally, the chances that the 5 (+1) gynandromorphs of the ambiguous type would have been positively diagnostic if they had developed from two nuclei were from 2:1 to 30:1.

6. The absence of any flies positively diagnostic for two nuclei does not give any more clue as to the frequency of binucleated eggs than that, if they occur at all, they are probably at most not much more frequent than one in 74,000 flies.

7. Although one half of all gynandromorphs that can be explained by simple single elimination (types a and b) can also be explained as derived from two nuclei (that is those of type b), the data from miscellaneous experiments, as well as the results of the critical experiment, leave no doubt that, with possibly a rare exception, those of type b have been derived by elimination. The conclusion is based on the 1:1 ratio of type b to type a; on the inconsistency, for binucleated eggs, of ratios between type b and certain other types of composite, notably autosomal mosaics; on such evidence for the XO constitution of the male region as the sterility of gynandromorphs having apparently normal male abdomens and the bobbed appearance of genetically bobbed male regions (*cf.* 13a); on the frequency of various kinds of single chromosomal elimination as shown by several different kinds of composites (*cf.* 11).

8. There is new evidence from various sources bearing on both interpretations of flies like the "special cases" that are explicable by multiple elimination from a non-disjunctional zygote or as derived from two nuclei. The discovery of autosomal mosaics has furnished genetic evidence that binucleated eggs probably sometimes occur in *Drosophila,* and there is the following evidence for multiple elimination:

a. A gynandromorph was observed by Hyde and Powell that had a female region and a mosaic male head, that is, two phenotypically different male regions. The fly can be explained by double elimination, but not on the binuclear theory.

b. Two mosaic females found in the critical experiment contained two X-chromosomes from the mother in one region and two X-chromosomes from the father and none from the mother in the other region. These cannot be accounted for as originating from two nuclei, but can be formally explained if a non-disjunctional egg was fertilized by non-disjunctional sperm and multiple elimination afterwards took place.

9. The ratios to be expected according to the different interpretations have been compared with findings recorded in all available data which though meager at least indicate the following results:

a. A number of types if derived by double elimination give ratios inter se that are fairly in accord with expectation. But if it is assumed that they developed from an egg with two nuclei, the ratios of these to other types from binucleated eggs are not as expected, and, for some of them, the additional assumption must be made that one of the two nuclei from which they originated was non-disjunctional.

b. Gynandromorphs explicable as derived from binucleated eggs or by triple elimination from non-disjunctional eggs have all been found to contain probably one crossover and one non-crossover chromosome (in one case two crossovers at different levels) which is characteristic of equational non-disjunction, and if they developed from non-disjunctional eggs, the distribution of chromosomes in the gynandromorphs is not different from expectation. But it is not apparent why corresponding gynandromorphs have not been found from reductional non-disjunctional eggs. (The phenotype of this type (h) has been found only in backcrosses where single elimination furnishes a simpler explanation.) The results are even farther from expectation on the binuclear hypothesis for the phenotype of the missing class would result from binucleated eggs when the nuclei contained the two original maternal X's, and this missing type is (excluding the ambiguous type b) the one to be expected most frequently from binucleated eggs in the absence of autosomal markings.

10. It appears from the critical experiment and from miscellaneous experiments that, although binucleated eggs do sometimes occur, they are not only far more rare than single eliminations but perhaps also more rare than multiple elimination from a non-disjunctional zygote.

11. Besides the two large classes of gynandromorphs that are produced by single elimination from a 2N zygote, there are further instances of single elimination:

a. an X-chromosome from a regular exceptional XXY zygote in high non-disjunctional stocks (non-disjunctional gynandromorph).
b. a Y-chromosome from an XXY zygote (Stern 1927*a*).
c. a IV-chromosome from a 2N zygote (Bridges 1921 *a* and *b*).
d. an X-chromosome from a 3X2A zygote.
e. an X-chromosome from a 3X3A zygote.
f. an arm of a III-chromosome (Stern 1927*b*).

The absence of certain kinds of composite which would be expected if a single large autosome were eliminated is probably due to inviability of the resulting combination of chromosomes; haplo-II and haplo-III individuals are known to be inviable (*cf*. 13c).

12. Elimination of a chromosome may be associated with:

a. the shape of a chromosome, as shown in "U"-stock by a high percentage of non-disjunctional males from heterozygous mothers and a still higher percentage from mothers homozygous for the U-shaped chromosome, and as shown also by relatively high frequency of gynandromorphs resulting from elimination of the curved chromosome.

b. some mutant change in a chromosome as "minute n" in piebald stock (Bridges 1925*a*).

13. New data give added evidence in regard to the following already known properties of gynandromorphs and show that similar relations hold for other composites.

a. Simple gynandromorphs breed sometimes as females but never as males, which is in accord with the expected XO constitution of the male region if it is formed by elimination, as shown by Morgan and Bridges (1919).

b. The XO constitution of the male region has been demonstrated by means of the character bobbed, which does not show in males because of the presence of its normal allelomorph in the Y-chromosome, but shows in the male region of gynandromorphs in bobbed stock first found by Stern (1926, 1927*a*).

c. Viability of gynandromorphs and of other composites is dependent on the viability of each of the component regions; an extensive and completely inviable region whether due to lethal genes or to unbalance of chromosomes has not been found associated with a viable region.

d. Fertility like other characters is independent in the two regions of gynandromorphs and other composites; there is some evidence that regional division other than that including the sexual apparatus is also concerned.

e. Vermilion was first shown by Sturtevant (1920) to be sometimes an exception in regard to independence of characters in gynandromorphs. There are new instances of red eye in a genetically vermilion XO male region of a gynandromorph that is heterozygous for vermilion in the female region. In other cases of XO constitution, however, vermilion is known to show, as in XO non-disjunctional males that carry vermilion and in gynandromorphs homozygous for vermilion that have one or both vermilion eyes included in the male region. One new gynandromorph is reported as showing vermilion in the male eyes when the female region was heterozygous. Vermilion showed in one eye of a mosaic male; the normal allelomorph and corresponding not vermilion eye were present in the other region. Two gynandromorphs with three different X-chromosomes having the normal allelomorph for vermilion in one chromosome, and a corresponding red eye in the male region, can be interpreted as having one crossover chromosome not carrying vermilion in the female region, or as homozygous for vermilion in the female region but phenotypically red.

BIBLIOGRAPHY

Anderson, E. G. 1924. X-rays and the frequency of non-disjunction in *Drosophila.* Papers of the Michigan Acad. Sci. Arts and Letters IV: 523–525.

———. 1925. The proportion of exceptions in the offspring of exceptional females from X-ray treatment in *Drosophila.* Papers of the Michigan Acad. Sci. Arts and Letters V: 355–366.

Bridges, C. B. 1913. Non-disjunction of the sex-chromosomes of *Drosophila.* Journ. Exp. Zool. XV: 587-606.

———. 1916. Non-disjunction as proof of the chromosome theory of heredity. Genetics I: 136.

———. 1921*a*. Proof of non-disjunction for the fourth of chromosome of *Drosophila melanogaster.* Science, n.s., LIII: 308.

———. 1921*b*. Genetical and cytological proof of non-disjunction of the fourth chromosome of *Drosophila melanogaster.* Proc. Nat. Acad. Sci. VII: 186–192.

———. 1921*c*. Triploid intersexes in *Drosophila melanogaster.* Science, n.s., LIV: 252–254.

———. 1922. The origin of variations in sexual and sex-limited characters. Am. Nat., LVI: 51–63.

———. 1925*a*. Elimination of chromosomes due to a mutant (minute-n) in *Drosophila melanogaster.* Proc. Nat. Acad. Sci., XI: 701–706.

———. 1925*b*. Haploidy in *Drosophila melanogaster.* Proc. Nat. Acad. Sci., XI: 706–710.

———, and T. M. Olbrycht. 1926. The multiple stock "X-ple" and its use. Genetics XI: 41–56.

Dexter, J. S. 1912. On coupling of certain sex-linked characters in *Drosophila.* Biol. Bull., XXIII: 183–194.

Doncaster, L. 1914. On the relations between chromosomes, sex-limited transmission, and sex determination in *Abraxas grossulariata.* Journ. Gen. IV: 1–22.

———. 1916. On some gynandromorphic specimens of *Abraxas grossulariata.* Proc. Cambridge Phil. Soc. XVIII: 227–229.

Duncan, F. N. 1915. A note on the gonads of gynandromorphs of *Drosophila ampelophila.* Am. Nat. XLIX: 455–456.

Hyde, R. R., and H. M. Powell. 1916. Mosaics in *Drosophila ampelophila.* Genetics I: 581–583.

Mavor, J. W. 1921*a*. Elimination of the X-chromosome from the egg of *Drosophila melanogaster* by X-rays. Proc. Soc. Exp. Biol. Med., XVIII: 303–304.

———. 1921*b*. On the elimination of the X-chromosome from the egg of *Drosophila melanogaster* by X-rays. Science, n.s., LIV: 277–279.

———. 1922. The production of non-disjunction by X-rays. Science, n.s., LV: 295–297.

———. 1923. X-rays and the sex chromosomes. Science, n.s., LVII: 503–504.

———. 1924*a*. The production of non-disjunction by X-rays. Journ. Exp. Zool., XXXIX: 381–432.

———. 1924*b*. Gynandromorphs from X-rayed mothers. Am. Nat., LVIII: 525–529.

Mohr, O. L. 1922. Cases of mimic mutations and secondary mutations in the X-chromosome of *Drosophila melanogaster.* Zeits. ind. Abst. Vererb. XXVIII: 1–22.

———. 1923. A genetic and cytological analysis of a section deficiency involving four units of the X-chromosome in *Drosophila melanogaster.* Zeits. ind. Abst. Vererb. XXXII: 108–232.

Morgan, L. V. 1922. Non-criss-cross inheritance in *Drosophila melanogaster.* Biol. Bull. XLII: 267–274.

———. 1925. Polyploidy in *Drosophila melanogaster* with two attached X-chromosomes. Genetics X: 148–178.

———. 1926. Correlation between shape and behavior of a chromosome. Proc. Nat. Acad. Sci., XII: 180–181.

Morgan, T. H. 1907. The cause of gynandromorphs in insects. Am. Nat. XLI: 715–718.

———. 1913. Heredity and sex. New York, p. 163.

———. 1914. Mosaics and gynandromorphs in *Drosophila.* Proc. Soc. Exp. Biol. and Med., XI: 171–172.

———. 1919. A demonstration of genes modifying the character "Notch." Carnegie Inst. Wash., Pub. No. 278, p. 384.

———. 1923. The physical basis of heredity. Philadelphia, pp. 189–192.

———. 1927. Exceptional classes of individuals in an experiment involving the bar locus of *Drosophila.* Hereditas, IX: 1–9.

MORGAN, T. H., and C. B. BRIDGES. 1913. Dilution effects and bi-colorism in certain eye-colors of *Drosophila*. Journ. Exp. Zool. XV: 450.

———, ———. 1919. The origin of gynandromorphs. Carnegie Inst. Wash. Pub. No. 278, 1–122.

———, A. H. STURTEVANT, H. J. MULLER, and C. B. BRIDGES. 1923. The mechanism of Mendelian heredity, revised ed. New York, pp. 116–124.

———, A. H. STURTEVANT, and C. B. BRIDGES. 1923. The constitution of the germ material in relation to heredity. Carnegie Inst. Washington, Year Book, No. 22, p. 286.

———, ———. 1925. Ditto. No. 24, 286–288.

———, C. B. BRIDGES, and A. H. STURTEVANT. 1925. The genetics of *Drosophila*. Bibliographia Genetica II: 144–152.

PAYNE, F., and MARTHA DENNY. 1922. A gynandromorph in *Drosophila melanogaster*. Am. Nat., LVI: 383.

SAFIR, S. R. 1920. Genetic and cytological examination of the phenomena of primary non-disjunction in *Drosophila melanogaster*. Genetics V: 459–487.

STERN, C. 1926. Vererbung im Y-chromosome von *Drosophila melanogaster*. Biol. Zentralbl. XLVI: 344–348.

———. 1927*a*. Ein genetischer und zytologischer Beweis für Vererbung im Y-chromosome von *Drosophila melanogaster*. Zeits. ind. Abst. Vererb., XLIV: 187–231.

———. 1927*b*. Über Chromosomenelimination bei der Taufliege. Die Naturwissenschaften XV: 740–746.

STURTEVANT, A. H. 1920. The vermilion gene and gynandromorphism. Proc. Soc. Exp. Biol. and Med., XVII: 70–71.

———. 1921*a*. Genetic studies on *Drosophila simulans*. II. Sex-linked group of genes. Genetics VI: 43–64.

———. 1921*b*. Genetic studies on *Drosophila simulans*. III. Autosomal genes. General discussion. Genetics VI: 179–207.

———. 1925. The effects of unequal crossing-over at the bar locus in *Drosophila*. Genetics X: 117–147.

———. 1927. The effects of the bar gene of Drosophila in mosaic eyes. Journ. Exp. Zool., XLVI: 493–498.

TOYAMA, K. 1906. Studies on hybridology of insects. I. On some silkworm crosses, with special reference to Mendel's law of heredity. Bull. Col. Agric., Tokyo Imperial University, VII.

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